BIO-TEK®

ELx800™

Absorbance Microplate Reader
Operator's Manual

Manual Update

7331000 Rev N2
July 2005

This **Manual Update** contains changes to the ELx800™ Operator's Manual.
These changes will be incorporated in the next full revision (Rev O) of the Manual.

Page 43, System Startup

Addition:

> **Important!** Some ELx800 readers have custom programmed software installed. Not all features of the software discussed in this Operator's Manual are available on custom instruments.

Please contact Bio-Tek's Technical Assistance Center at 800-242-4685 if you have any questions about the software on your reader.

Page 119, Liquid Test 1

Recommendation:

After pipetting the diluted test solution into the microplate (step 3) and *before* reading the plate, we strongly recommend shaking the plate for four minutes. This will allow any air bubbles in the solution to settle and the meniscus to stabilize. If a plate shaker is not available, wait 20 minutes after pipetting the diluted test solution before reading the plate.

BIO-TEK®

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ELx800™

Absorbance Microplate Reader
Operator's Manual

October 2004

Part Number 7331000
Revision N
Bio-Tek® Instruments, Inc.

Notices

Bio-Tek® Instruments, Inc.

Highland Park, P.O. Box 998

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Document Conventions

This manual uses the following typographic conventions:

Example	Description
	This icon calls attention to important **safety** notes.
Warning!	A **Warning** indicates the potential for bodily harm and tells you how to avoid the problem.
Caution	A **Caution** indicates potential damage to the instrument and tells you how to avoid the problem.
DEFINE	Text in **COURIER** font represents menu options as they appear on the instrument's display.
Note:	**Bold** text is primarily used for emphasis.
	This icon calls attention to important information.

Revision History

Revision	Date	Changes
A	3/95	First Release
B	7/95	Generic
C	9/95	Add Errata sheet
D	10/95	Incorporate Errata: cal plate PN cover text to UV
E	11/96	Added Panel Assay info. Added TVar parameter. Added Appendix D to include all reports.
F	2/97	Added Notes including info on 384-well format. Updated Reuse of Standard Curves and Panel sections. Added Appendix E – KcJr for 384-well format.
G	03/98	Added Liquid Test 1 and 2 to Chapter 4 –Performance Verification. Added Appendix F – Comparison, to show the differences between the ELx800 and the EL800. Added printer information. Changed plate type information to include 384-well and 72-/96-well Terasaki plate formats. Added reference to ***Quick Read*** on display.
H	09/98	Modified Appendix B - Computer Control. Updated Elx800 and EL800 model comparison table in Appendix F.
I	2/99	Incorporated Manual Updates. Changed European addresses.
J	9/99	Changed printer-compatible description. Changed the number of samples necessary if there aren't controls, standards or blanks defined. Corrected the number of stored plates to 8. Corrected the positions for re-use of standard curves. Removed the "M" command from Appendix B- Computer Control.
K	1/00	Added Liquid Test 3 to test 340 nm filters. Updated screens in Chapter 3. Updated chart in Appendix F.
L	5/03	Preface: - Updated contact information in Notices (page iii). - Added Document Conventions (page v). - Corrected errors in Safety Symbol text (page ix). Removed "screwdriver" from Package Contents, and added "Bio-Tek QC Check Solutions for 405 nm" to Optional Accessories list (page 1-7). Removed **About This Manual** section (page 1-5). Updated contact information in Technical Support (Chapter 1). Modified appearance of display screens throughout. Revised lamp alignment section in Chapter 2.

Revision History (Continued)

L	5/03 (Cont.)	Chapter 4: - Changed title to "Performance Verification and IQ/PQ/OQ Tests." - Added IQ/PQ/OQ test procedure information. - Clarified procedures for liquid tests. Replaced outdated Appendix E with instructions for using KCjunior to read 384-well microplates. Added new Appendix G with two examples of assay kit instructions and directions for programming an assay. Made editorial and formatting changes throughout.
M	9/03	Preface: - Updated Warnings section (pages vii and viii). - Updated Electromagnetic Compatibility section (page x). - Added “Consult instructions for use” and “In vitro diagnostic medical device” safety symbols (page xii). - Expanded Intended Use Statement (page xiii). Added Absorbance Test Plate to Optional Accessories list (Chapter 1, page 1-8). Changed callout in Figure 2-2 from “Place unit in bag” to “Unit in plastic bag” (Chapter 2, page 2-2). Added KC4 startup information to Appendix E. Removed references to ELx800UV and EL800UV from Appendix F. Standardized the presentation of significant digits throughout. Changed “Abs” to “OD” throughout.
N	10/04	Restructured manual according to new template. Removed references to “General Formula” throughout. Updated Chapter 4, Performance Verification and IQ-OQ-PQ Procedures. Added new Chapter 5, Maintenance and Decontamination. Added new Chapter 6, Troubleshooting and Error Codes. Removed Computer Control portion of Appendix A; renamed Appendix to “Controlling the Reader With KCjunior™ or KC4™.” Added new Appendix B, Using 384-Well Geometry. Updated sample reports in Appendix C, Report Format (formerly Appendix D). Removed previous Appendix A, Decontamination. Removed previous Appendix C, Error Codes. Added new Appendix E, Instructions for Programming a New Assay.

Intended Use Statement

The intended use of this instrument is dependent on the instrument's rear panel label. If there is an IVD label, then the instrument may be used for clinical, research and development, or other nonclinical purposes. If there is no such label, then the instrument may **only** be used for research and development, or for other nonclinical purposes.

Quality Control

It is considered good laboratory practice to run laboratory samples according to instructions and specific recommendations included in the assay package insert for the test to be conducted. Failure to conduct Quality Control checks could result in erroneous test data.

Repackaging and Shipping

If you need to ship the instrument to Bio-Tek for service or repair, contact Bio-Tek for a Return Materials Authorization (RMA) number, and be sure to use the original packing. Other forms of commercially available packing are not recommended and can void the warranty. If the original packing materials have been damaged or lost, contact Bio-Tek for replacement packing.

Warnings

General

- Operate the instrument on a flat surface away from excessive humidity.

Readers

- **Excessive Ambient Light:** Bright sunlight or strong incandescent light can reduce the linear performance range of the instrument.
- **Dust:** Measurement values may be affected by extraneous particles (such as dust) in the microplate wells. A clean work area is necessary to ensure accurate readings.
- When operated in a safe environment according to the instructions in this document, there are no known hazards associated with the instrument. However, the operator should be aware of certain situations that could result in serious injury; these may vary depending on the instrument model.

Hazards and Precautions

Hazards

Warning! Power Rating. The instrument's power supply or power cord must be connected to a power receptacle that provides voltage and current within the specified rating for the system. Use of an incompatible power receptacle may produce electrical shock and fire hazards.

Warning! Electrical Grounding. Never use a two-prong plug adapter to connect primary power to the external power supply. Use of a two-prong adapter disconnects the utility ground, creating a severe shock hazard. Always connect the power cord directly to a three-prong receptacle with a functional ground.

Warning! Internal Voltage. Always turn off the power switch and unplug the power cord before cleaning the outer surface of the instrument.

Warning! Potential Biohazards. Some assays or specimens may pose a biohazard. Adequate safety precautions should be taken as outlined in the assay's package insert. Always wear safety glasses and appropriate protective equipment, such as chemically resistant rubber gloves and apron.

Warning! Liquids. Avoid spilling liquids on the instrument; fluid seepage into internal components creates a potential for shock hazard. Wipe up all spills immediately. Do not operate the instrument if internal components have been exposed to fluid.

Warning! Software Quality Control. The operator must follow the manufacturer's assay package insert when modifying software parameters and establishing reading or wash methods, using the instrument's onboard software. **Failure to conduct quality control checks could result in erroneous test data.**

Warning! Reader Data Reduction Protocol. For readers with onboard assay software, the software will flag properly defined controls when they are out of range. The software will present the data with the appropriate error flags for the operator to determine control and assay validity. For readers operated via computer control, no limits are applied to the raw absorbance data. All information exported via computer control must be thoroughly analyzed by the operator.

Precautions

The following precautions are provided to help avoid damage to the instrument:

Caution: Service. Only Bio-Tek authorized service personnel should service the instrument. Only qualified technical personnel should perform troubleshooting and service procedures on internal components.

Caution: Environmental Conditions. Do not expose the system to temperature extremes. For proper operation, ambient temperatures should remain between 15°-40°C. Performance may be adversely affected if temperatures fluctuate above or below this range. Storage temperature limits are broader.

Caution: Sodium Hypochlorite. Do not expose any part of the instrument to the recommended diluted sodium hypochlorite solution (bleach) for more than 20 minutes. Prolonged contact may damage the instrument surfaces. Be certain to rinse and thoroughly wipe all surfaces.

Caution: Warranty. Failure to follow preventive maintenance protocols may **void the warranty.**

Caution: Disposal. This instrument contains printed circuit boards and wiring with lead solder. Dispose of the instrument according to Directive 2002/96/EC, "on waste electrical and electronic equipment (WEEE)."

Based on the testing described below and information contained herein, this instrument bears the CE mark.

Directive 89/336/EEC:Electromagnetic Compatibility

Emissions - CLASS A

IEC 61326-1:2002

EN 55022:2000 Class A, CISPR 16-1:1993, CISPR 16-2:1999

Immunity

IEC 61326-1:2000

EN 61000-4-2: Electrostatic Discharge

EN 61000-4-3: Radiated EM Fields

EN 61000-4-4: Electrical Fast Transient/Burst

EN 61000-4-5: Surge Immunity

EN 61000-4-6: Conducted Disturbances

EN 61000-4-11: Voltage Dips, Short Interruptions and Variations

Directive 73/23/EEC Low Voltage

EN 61010-1 (2001): "Safety requirement for electrical equipment for measurement, control and laboratory use. Part 1, General requirements".

Directive 2002/96/EC: Waste Electrical and Electronic Equipment

Disposal Notice

This instrument contains printed circuit boards and wiring with lead solder. Dispose of the instrument according to Directive 2002/96/EC, "on waste electrical and electronic equipment (WEEE)."

Directive 98/79/EC: *In Vitro* Diagnostics

- Product registration with competent authorities
- Traceability to the U.S. National Institute of Standards and Technology (NIST):
  - Optical density measurements, and if equipped, incubator temperature readings, are traceable to NIST.

Specific data for a particular serial number is available on request from Bio-Tek Instruments. See page xi for contact information.

Electromagnetic Interference and Susceptibility

USA FCC CLASS A

Warning: Changes or modifications to this unit not expressly approved by the manufacturer could void the user's authority to operate the equipment.

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules.

These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. Like all similar equipment, this equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause interference, in which case the user will be required to correct the interference at his/her expense.

Canadian Department of Communications Class A

This digital apparatus does not exceed Class A limits for radio emissions from digital apparatus set out in the Radio Interference Regulations of the Canadian Department of Communications.

Le present appareil numerique n'emet pas du bruits radioelectriques depassant les limites applicables aux appareils numerique de la Class A prescrites dans le Reglement sur le brouillage radioelectrique edicte par le ministere des Communications du Canada.

User Safety

This device has been type tested by an independent laboratory and found to meet the requirements of the following:

North America

- **Canadian Standards Association CAN/CSA C22.2 No. 1010.1-92**

  "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements"

- **UL 61010A-1, 1st Edition**

  "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements"

International

- **EN 61010-1:2001**

  "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements"

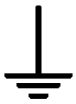
Safety Symbols

Some of the following symbols will appear on the instrument.

Alternating current
Courant alternatif
Wechselstrom
Corriente alterna
Corrente alternata

Direct current
Courant continu
Gleichstrom
Corriente continua
Corrente continua

Both direct and alternating current
Courant continu et courant alternatif
Gleich - und Wechselstrom
Corriente continua y corriente alterna
Corrente continua e corrente alternata



Earth ground terminal
Borne de terre
Erde (Betriebserde)
Borne de Tierra
Terra (di funzionamento)

Protective conductor terminal
Borne de terre de protection
Schutzleiteranschluss
Borne de tierra de protección
Terra di protezione

On (Supply)
Marche (alimentation)
Ein (Verbindung mit dem Netz)
Conectado
Chiuso

Off (Supply)
Arrêt (alimentation)
Aus (Trennung vom Netz)
Desconectado
Aperto (sconnessione dalla rete di alimentazione)

Caution (refer to accompanying documents)
Attention (voir documents d'accompanement)
Achtung siehe Begleitpapiere
Atención (vease los documentos incluidos)
Attenzione, consultare la doc annessa

Warning, risk of electric shock
Attention, risque de choc électrique
Gefährliche elektrische Shlag
Precaución, riesgo de sacudida eléctrica
Attenzione, rischio di scossa elettrica

Warning, risk of crushing or pinching
Attention, risque d'écrasement et pincement
Warnen, Gefahr des Zerquetschens und Klemmen
Precaución, riesgo del machacamiento y sejeción
Attenzione, rischio di schiacciare ed intrappolarsi

Warning, hot surface
Attention, surface chaude
Warnen, heiße Oberfläche
Precaución, superficie caliente
Attenzione, superficie calda

Consult instructions for use
Consulter la notice d'emploi
Gebrauchsanweisung beachten
Consultar las instrucciones de uso
Consultare le istruzioni per uso

***In vitro* diagnostic medical device**
Dispositif médical de diagnostic *in vitro*
Medizinisches *In-Vitro*-Diagnostikum
Dispositivo médico de diagnóstico *in vitro*
Dispositivo medico diagnostico *in vitro*

Separate collection for electrical and electronic equipment
Les équipements électriques et électroniques font l'objet d'une collecte sélective
Getrennte Sammlung von Elektro- und Elektronikgeräten
Recogida selectiva de aparatos eléctricos y electrónicos
Raccolta separata delle apparecchiature elettriche ed elettroniche

Warranty

This Warranty is limited and applies only to new products, except for computer-based software, which is covered under a separate Warranty Policy, manufactured by Bio-Tek Instruments, Inc. (“Bio-Tek”). Bio-Tek makes no warranty whatsoever regarding the condition of used products.

Bio-Tek warrants the instrument (hereinafter collectively referred to as “Products” or “Product”) for a period of one (1) year from the original purchase date against defective materials or workmanship. This Warranty is limited to the original purchaser (the “Purchaser”) and cannot be assigned or transferred. All claims under this Limited Warranty must be made in writing to Bio-Tek, Attention: Service Department. Purchaser must ship the Product to Bio-Tek, postage pre-paid. Bio-Tek shall either repair or replace with new or like new, at its option and without cost to the Purchaser, any Product which in Bio-Tek’s sole judgment is defective by reason of defects in the materials or workmanship.

This Warranty is VOID if the Product has been damaged by accident or misuse, or has been damaged by abuse or negligence in the operation or maintenance of the Product, including without limitation unsafe operation, operation by untrained personnel, and failure to perform routine maintenance. This Warranty is VOID if the Product has been repaired or altered by persons not authorized by Bio-Tek, or if the Product has had the serial number altered, effaced, or removed. This Warranty is VOID if the Product has not been connected, installed, or adjusted strictly in accordance with written directions furnished by Bio-Tek. Batteries, fuses, light bulbs, and other “consumable” items used in any of the Products are not covered by this Warranty. Software utilized in conjunction with any of the Products is not covered by the terms of this Warranty but may be covered under a separate Bio-Tek software warranty.

We will continue to stock parts for a minimum period of five (5) years after the manufacture of any equipment has been discontinued. Parts shall include all materials, charts, instructions, diagrams, and accessories that were furnished with the standard models.

THIS WARRANTY CONTAINS THE ENTIRE OBLIGATION OF BIO-TEK INSTRUMENTS, INC., AND NO OTHER WARRANTIES, EXPRESSED, IMPLIED, OR STATUTORY ARE GIVEN. PURCHASER AGREES TO ASSUME ALL LIABILITY FOR ANY DAMAGES AND/OR BODILY INJURY OR DEATH THAT MAY RESULT FROM THE USE OR MISUSE OF ANY EQUIPMENT OR INSTRUMENT BY THE PURCHASER, HIS EMPLOYEES, AGENTS, OR CUSTOMERS, OTHER THAN THE EXPRESS WARRANTY CONTAINED HEREIN. WE SHALL NOT BE RESPONSIBLE FOR ANY DIRECT OR CONSEQUENTIAL DAMAGES OF ANY KIND. THIS WARRANTY SHALL NOT BE CHANGED OR MODIFIED IN ANY WAY WITHOUT THE EXPRESS WRITTEN PERMISSION OF AN OFFICER OF BIO-TEK INSTRUMENTS, INC.

Registration Card

Once the instrument has been set up and is running successfully, please take a moment to fill out and mail the postage-paid Warranty Registration card. By sending in the registration card, you will be assured of receiving prompt information on product enhancements.

Chapter 1

Introduction

This chapter introduces the ELx800 Automated Microplate Reader and describes its hardware and software features. Also included is contact information if technical assistance is needed.

Introducing the ELx800 Automated Microplate Reader

The ELx800 Automated Microplate Reader is a single-channel reader-assay system, designed to automatically perform endpoint analysis for a variety of ELISA-based applications. The reader can measure the optical density of solutions in 6-, 12-, 24-, 48-, or 96-well microplates. The "NB" reader models can measure 384-well microplates and 60-, 72-, 96-well Terasaki trays as well as 6-, 12, 24-, 48-, or 96-well plates. The reader features superior optical specifications, with an extended dynamic range of up to 3.000 absorbance units in some read modes. The wavelength range is from 400 nm to 750 nm. "UV" instruments have an extended range from 340 nm to 750 nm. Kinetic analysis can be performed using computer control (e.g., via KC4™ or KCjunior™ running on a host PC).

Assay definitions (consisting of protocols, templates and formulas) and the data they produce are managed by an onboard processor, via a 2-line x 24-character LCD screen and membrane switch. Data can be stored onboard, printed, and/or uploaded to controlling software on a host PC. The ELx800 is designed to serve as a stand-alone system, or as part of a larger laboratory data network, sending, receiving, and manipulating assay data as needed.

The ELx800 is backed by a superior support staff. If the ELx800 ever fails to work perfectly, please call the Technical Assistance Center, or visit Bio-Tek's Web site. Refer to the end of this chapter for contact information.

Hardware Features

The ELx800's hardware features include:

- Single optics channel
- Wavelength range of 400-750 nm
    - ELx800UV model provides extended wavelength range of 340 to 750 nm
- A 5-position filter wheel
- A 2-line x 24-character LCD display
- A membrane keypad with alphanumeric keys
- X-Y carrier movement
- Capability of reading 6-, 12-, 24-, 48- and 96-well microplates.
    - ELx800NB model extends read capability to include 384-well and 60-/72-/96-well Terasaki microplates.
- External 24-volt power supply, which runs on 100 to 240 V~ ± 10.0% @ 50 to 60 Hz
- One serial COM port, 25-pin male connector
- One parallel port, 25-pin female connector

Software Features

- An easy-to-use, menu-driven interface
- Endpoint calculations
- Curve Fitting, with 4-parameter, cubic, quadratic, linear, 2-P, cubic-spline, and point-to-point methods
- Transformation and Formula calculations for more complex mathematical operations, including validations
- Up to 75 assays can be programmed into memory and recalled instantly
- Up to 10 results can be stored in memory

Package Contents

The contents of the ELx800 package include:

- Microplate Reader
- Power Cord (part number varies according to country of use)
- Power Supply
- Filter wheel with 4 standard filters: 405 nm, 450 nm, 490 nm, 630 nm and one blank filter. The UV model includes a 340 nm filter.
- Operator's Manual (PN 7331000) and Registration Card
- Printer Cable (PN 71072)
- Dust Cover (PN 7332040)
- RS-232 Serial Cable (PN 75053)
- Unpacking instructions (PN 7271003); packing instructions (PN 7271012)
- Shipping document kit (PN 94075), including Warranty Registration Card, Certificate of Compliance and Calibration, and FDA Certification Form
- Set of shipping materials (PN 7273000)
- Declaration of Conformity (PN 7271004)
- Sample UV transparent plates and literature

Optional Accessories

- Patented Bio-Cell™ Quartz vessel: to obtain 1 cm fixed pathlength absorbance measurements (PN 7272051)
- Bio-Cell adapter plate assembly: can contain up to 8 Bio-Cells (PN 7270512)
- Lamp for ELx800NB, ELx800RNB, ELx800UV, ELx800RUV (PN 7330516)
- Lamp for ELx800 and ELx800R (PN 7330513)
- ELx800 filters (PN 7334---, plus wavelength)
    - 340 (UV model only), 405, 415, 450, 490, 515, 540, 550, 562, 570, 590, 595, 600, 620, 630, 650, 660, 690, 750
- Terasaki plate adapter for 60-, 72-, and 96-well Terasaki plates for ELx800NB and ELx800RNB (PN 7330531)
- Absorbance Test Plate (PN 7260522)
- Epson LX-300 printer or equivalent (110V only) (PN 97145)
- Sheet feeder for Epson LX-300+ (PN 97144)
- HP DeskJet printer (PN 97152)
- KC4™ Software:
    - KC4 Reader Control and Data Analysis Software (PN 5290512)
    - KC4 Signature (21 CFR Part 11 Compliant Version) (PN 5290510)
    - KC4 Signature Upgrade (from v2.7 or lower) (PN 5290511)
    - KC4 Signature Upgrade (from v3.0) (PN 5290508)
    - KC4 Upgrade (from v2.7 or lower) (PN 5290514)
- KCjunior™ Software:
    - KCjunior Software Package (PN 5270501)
    - KCjunior Upgrade (PN 5270508)
- Delta-Soft Macintosh Software (PN 3070145)
- ELx800 Qualification Package (PN 7330538)

Specifications

Standard Model

Wavelength Range: 400 to 750 nm

Filters: 10 nm half-bandwidth interference filters.

User-accessible filter wheel. Up to 5 filters may be installed on the instrument at one time.

Filters supplied: 405 nm, 450 nm, 490 nm and 630 nm.

- The following specifications apply to 96-well, flat- or round-bottom plates, single-wavelength measurements with a **50-second read** (normal read mode).

Absorbance Measurement Range:	0.000 to 3.000 OD
Accuracy:	± 1.0% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm
Linearity:	± 1.0% from 0.000 to 2.000 OD at 405 nm
	± 3.0% from 2.000 OD to 3.000 OD @ 450 nm
Repeatability (STD):	± 0.5% ± 0.005 OD from 0.000 to 2.000 OD @ 405 nm

- The following specifications apply to 96-well, flat- or round-bottom plates, single-wavelength measurements with a **30-second read** (rapid read mode).

Absorbance Measurement Range:	0.000 to 3.000 OD
Accuracy:	± 2.0% ± 0.020 OD from 0.000 to 2.000 OD @ 405 nm
Linearity:	± 2.0% from 0.000 to 2.000 OD @ 405 nm
Repeatability (STD):	± 1.0% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm

- **Light Source:** Tungsten gas-filled bulb
- **Dimensions:** 42 cm x 38 cm x 18 cm (16.5" deep x 15" wide x 7" tall)
- **Weight:** 8 kg (18.5 lb. Maximum)
- **Environment:** Operating temperature 18° to 40°C
- **Humidity:** 10% to 85% noncondensing
- **Power Supply:** Input 100 to 240 V~ ± 10.0% @ 50 to 60 Hz
  Output +24 VDC, 2.1 A

Ultraviolet/UV Model

The following specifications apply to 96-well, flat- or round-bottom plates.

Wavelength Range:	340 to 750 nm
Filters:	10 nm half-bandwidth interference filters. User-accessible filter wheel. Up to 5 filters may be installed on the instrument at one time. Filters supplied: 340 nm, 405 nm, 450 nm, 490 nm and 630 nm.

- **Optical specifications** for the 400 to 750 nm range (**50-second read** in normal read mode):

Absorbance Measurement Range:	0.000 to 3.000 OD
Accuracy:	± 1.0% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm
Linearity:	± 1.0% from 0.000 to 2.000 OD @ 405 nm ± 3.0% from 2.000 to 3.000 OD @ 450 nm
Repeatability (STD):	± 0.5% ± 0.005 OD from 0.000 to 2.000 OD @ 405 nm

- **Optical specifications** for the 340 to 400 nm range **(50-second read** in normal read mode**):**

Absorbance Measurement Range:	0.000 to 3.000 OD
Accuracy:	± 2.0% ± 0.010 OD from 0.000 to 2.000 OD @ 340 nm
Linearity:	± 2.5% from 0.000 to 2.000 OD @ 340 nm
Repeatability (STD):	± 1.5% ± 0.005 OD from 0.000 to 2.000 OD @ 340 nm

- **Optical specifications** for the 400 to 750 nm range **(30-second read** in rapid read mode**):**

Absorbance Measurement Range:	0.000 to 3.000 OD
Accuracy:	± 2.0% ± 0.020 OD from 0.000 to 2.000 OD @ 405 nm
Linearity:	± 2.0% from 0.000 to 2.000 OD @ 405 nm
Repeatability (STD):	± 1.0% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm

- Optical specifications for the 340 to 400 nm range (30-second read):

Absorbance Measurement Range:	0.000 to 3/-*.000 OD
Accuracy:	± 2.5% ± 0.020 OD from 0.000 to 2.000 OD @ 340 nm
Linearity:	± 2.5% from 0.000 to 2.000 OD @ 340 nm
Repeatability (STD):	± 2.0% ± 0.010 OD from 0.000 to 2.000 OD @ 340 nm

Narrow Beam/NB Model

Filters:	10 nm half-bandwidth interference filters. User-accessible filter wheel. Up to 5 filters may be installed on the instrument at one time. Filters supplied: 405 nm, 450 nm, 490 nm and 630 nm.

The following specifications apply to 96-well, flat- or round-bottom plates with a **50-second read** (normal read mode).

Absorbance Measurement Range:	0.000 to 3.000 OD
Accuracy:	± 1.0% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm
Linearity:	± 1.0% from 0.000 to 2.000 OD @ 405 nm ± 3.0% from 2.000 to 3.000 OD @ 450 nm
Repeatability (STD):	± 0.5% ± 0.005 OD from 0 to 2.000 OD @ 405 nm

The following specifications apply to 96-well, flat- or round-bottom plates with a **30-second read** (rapid read mode).

Absorbance Measurement Range:	0.000 to 3.000 OD
Accuracy:	± 2.0% ± 0.020 OD from 0.000 to 2.000 OD @ 405 nm
Linearity:	± 2.0% from 0.000 to 2.000 OD @ 405 nm
Repeatability (STD):	± 1.0% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm

The following specifications apply to a 384-well plate. The minimum volume of fluid in a well to obtain the specified performance is 80 microliters. The specifications apply to the dual wavelength mode of read only.

Absorbance Measurement Range:	0.000 to 3.000 OD
Normal Read Mode:	Dual wavelength (4 minutes, 45 seconds)
Accuracy:	± 2.0% ± 0.020 OD from 0.000 to 2.000 OD @ 405 nm
Linearity:	± 2.5% from 0.000 to 2.000 OD @ 405 nm
Repeatability (STD):	± 1.5% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm
Rapid Read Mode:	Dual wavelength (3 minutes, 25 seconds)
Accuracy:	± 2.5% ± 0.020 OD from 0.000 to 2.000 OD @ 405 nm
Linearity:	± 2.5% from 0.000 to 2.000 OD @ 405 nm
Repeatability (STD):	± 2.0% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm

Technical Support

Bio-Tek's ELx800™ Absorbance Microplate Reader is backed by a superior support staff. If the reader ever fails to work perfectly, please contact Bio-Tek's Technical Assistance Center (TAC).

Whichever method of contact you choose, please be prepared to provide the following information:

- Product name and serial number.
- The software configuration information. To locate this information, press **UTIL** ➔ **TESTS** ➔ **CHKSUM** from the reader's Main Menu.
- The specific steps that reproduce your problem.
- Any error codes displayed on the screen (descriptions of error codes are available in **Chapter 7**).
- A daytime phone number.
- Your name and company information.
- A fax number and/or e-mail address, if available.

If you need to return the reader to Bio-Tek for service, contact Bio-Tek for a Return Materials Authorization number (RMA), repackage the reader properly (see **Chapter 3**, **Installation**), and ship the instrument to Bio-Tek at the shipping address listed on the following page.

Phone Support

You can telephone the Technical Assistance Center between 8:30 AM and 5:30 PM Eastern Standard Time (EST), Monday through Friday, excluding holidays.

Bio-Tek Instruments' Main Number: (802) 655-4040

Technical Assistance Center: (800) 242-4685

Electronic Communication

Electronic communication is available via the following:

E-Mail: tac@biotek.com

Internet Site: www.biotek.com

Facsimile Support

You may send a fax with your questions or requests for help 24 hours a day to the following number:

Technical Assistance Center: (802) 655-3399

Written Communication

If you prefer, you may write a letter with your comments and send it to:

Bio-Tek Instruments, Inc.
Technical Assistance Center
P.O. Box 998, Highland Park
Winooski, Vermont 05404-0998 USA

Shipping Address

Ship instruments that need repair or service to Bio-Tek at the following address:

Bio-Tek Instruments, Inc.
Technical Assistance Center
100 Tigan Street
Highland Park
Winooski, Vermont 05404 USA

Chapter 2

Installation

This chapter includes instructions for unpacking and setting up the ELx800 and instructions for connecting printers and/or serial devices.

Unpacking and Inspecting the ELx800™

Important! Save <u>all</u> packaging materials. If the ELx800™ is shipped to the factory for repair or replacement, it must be carefully repackaged, according to the instructions on pages 30 through 31, using the original packing materials (PN 7332062). The individual packaging materials contained in PN 7332062 are listed in the table below. Using other forms of commercially available packing materials, or failure to follow the repackaging instructions may **void your warranty**. If the original packing materials have been damaged, replacements are available from Bio-Tek.

The ELx800 and its accessories are securely packaged inside custom-designed shipping materials. This packaging should protect the instrument from damage during shipping. Inspect the shipping box, packaging, instrument, and accessories for signs of damage.

If the reader is damaged, notify the carrier and your manufacturer's representative. Keep the shipping cartons and packing material for the carrier's inspection. The manufacturer will arrange for repair or replacement of your instrument immediately, before the shipping-related claim is settled.

Refer to the unpacking instructions and **Figures 1** through **4** on the following pages when removing the instrument and its accessories from the shipping container.

See **Repackaging and Shipping the ELx800** at the end of this chapter for complete shipping instructions.

Packing Materials	PN
Outer shipping container	7332036
Inner shipping container (accessories)	7330002
Left foam end caps	7332060
Right foam end caps	7332061
2-Mil poly bag (ELx800)	98085
8 ½" x 11" bubble bag (power supply)	91083

Required Tools	PN
Slotted and Phillips-head screwdrivers	98145

Unpacking the Instrument and Its Accessories

1. Carefully open the top of the box, and remove the power supply shelf (**Figure 1**).
2. Remove the top foam end caps, manual, and Declaration of Conformity.

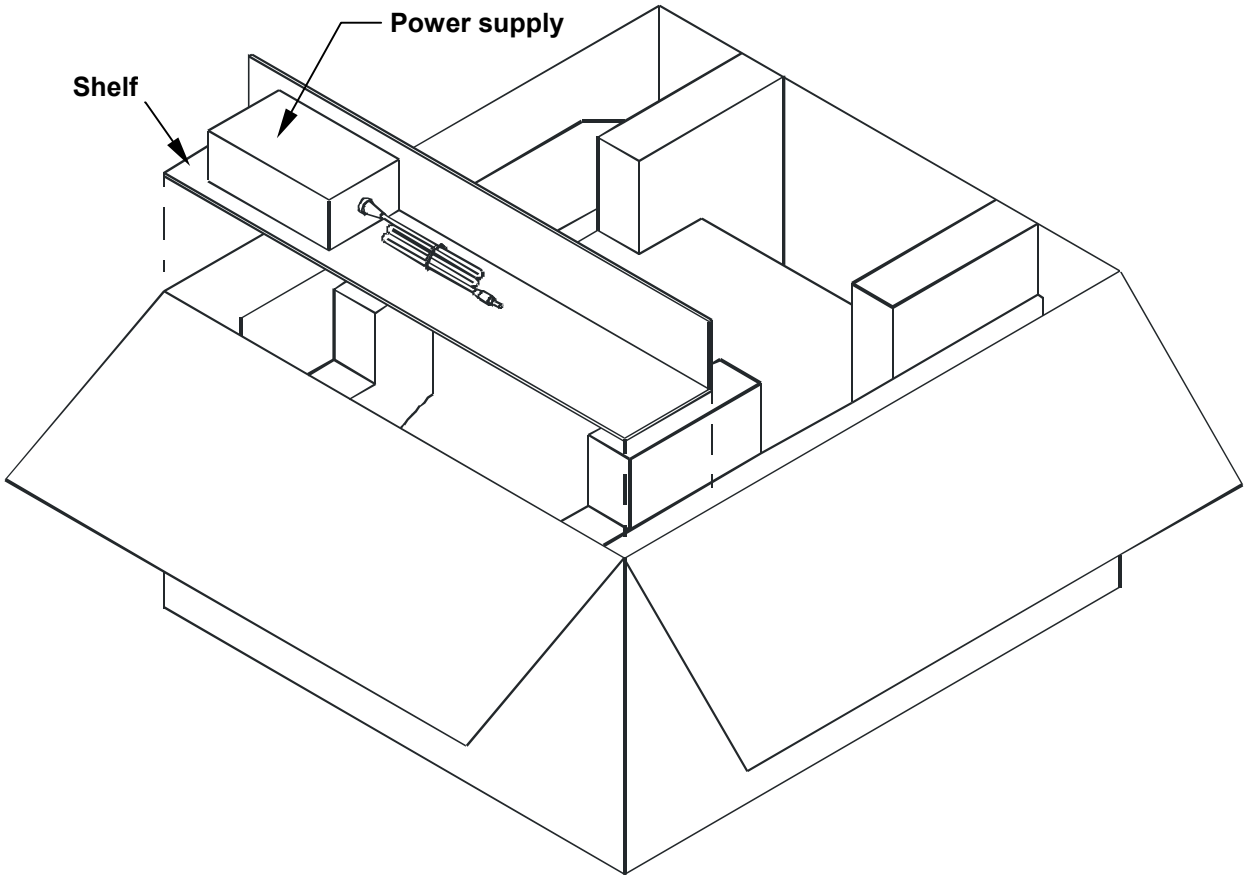


Figure 1: Removing the power supply and shelf

3. Lift the reader out of the box and remove the foam end caps (**Figure 2**). Place the reader on a level surface and remove the instrument from the plastic bag.

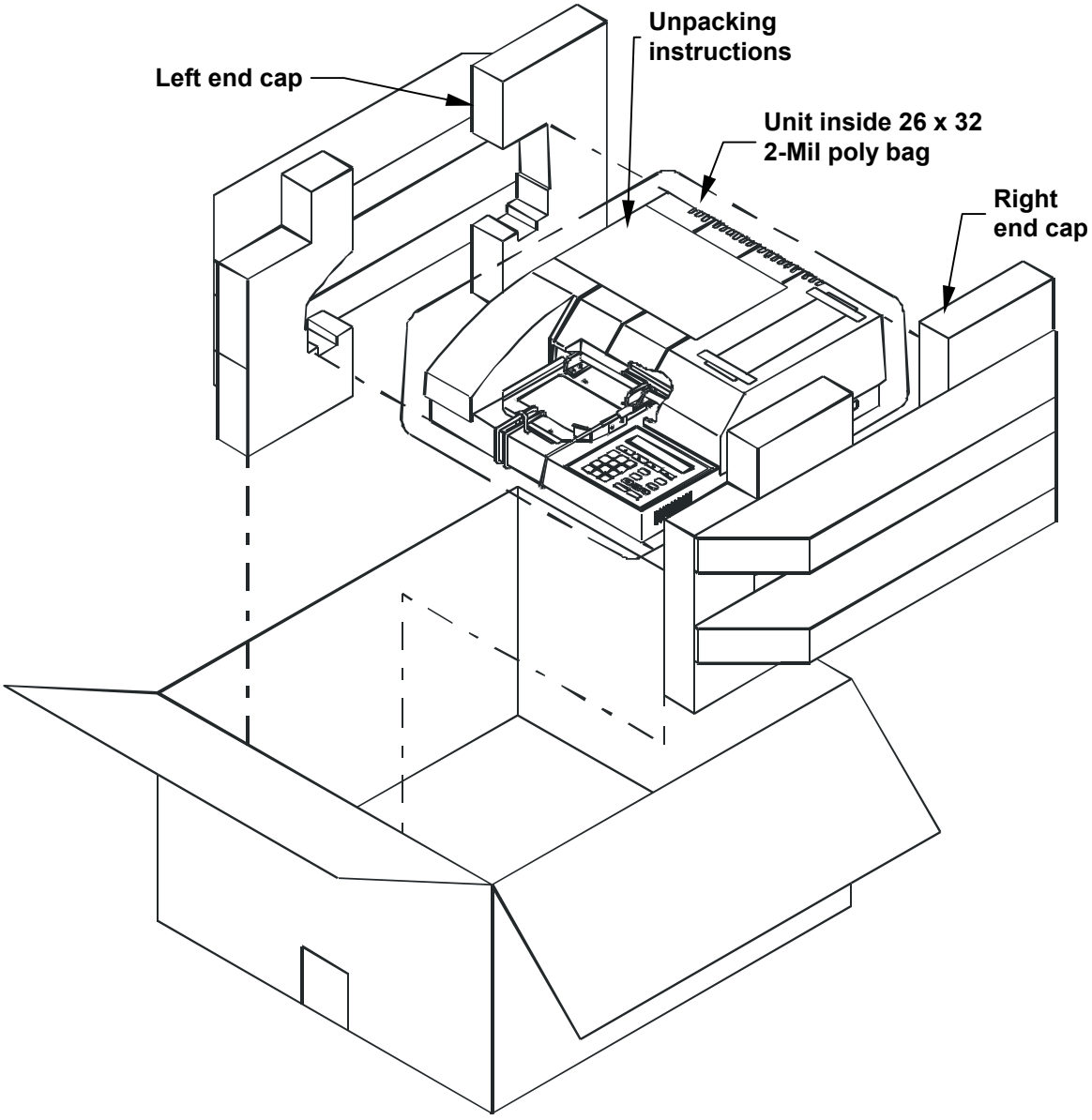


Figure 2: Removing the foam end caps

4. Carefully turn the ELx800 upside down on a level surface.
5. Use the slotted screwdriver to remove the four screws from the top cover mounting (**Figure 3**) and shipping straps from the bottom of the instrument. Set aside these screws.

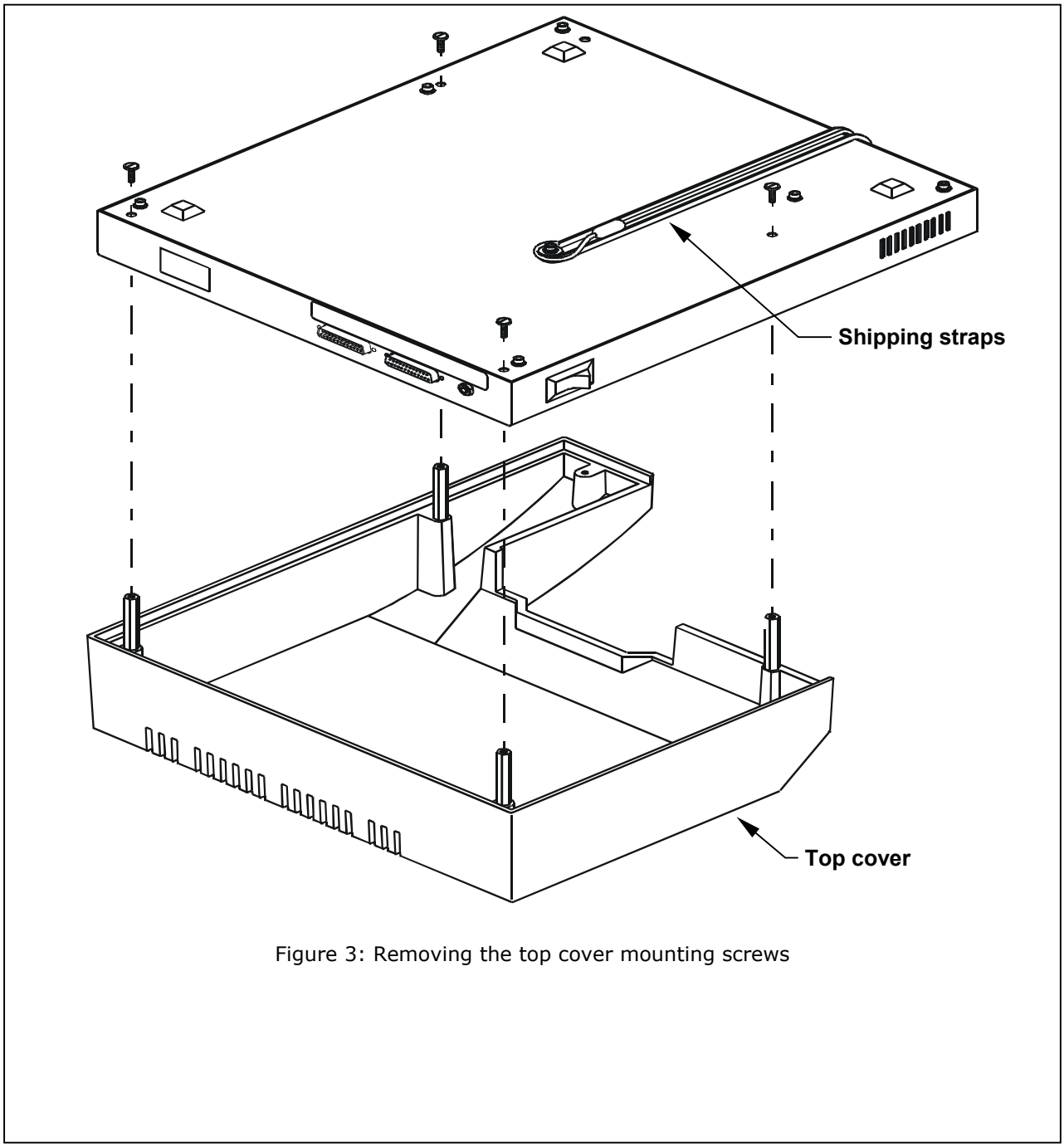


Figure 3: Removing the top cover mounting screws

6. Turn the instrument right side up.
7. Remove the shipping block (**Figure 4**).

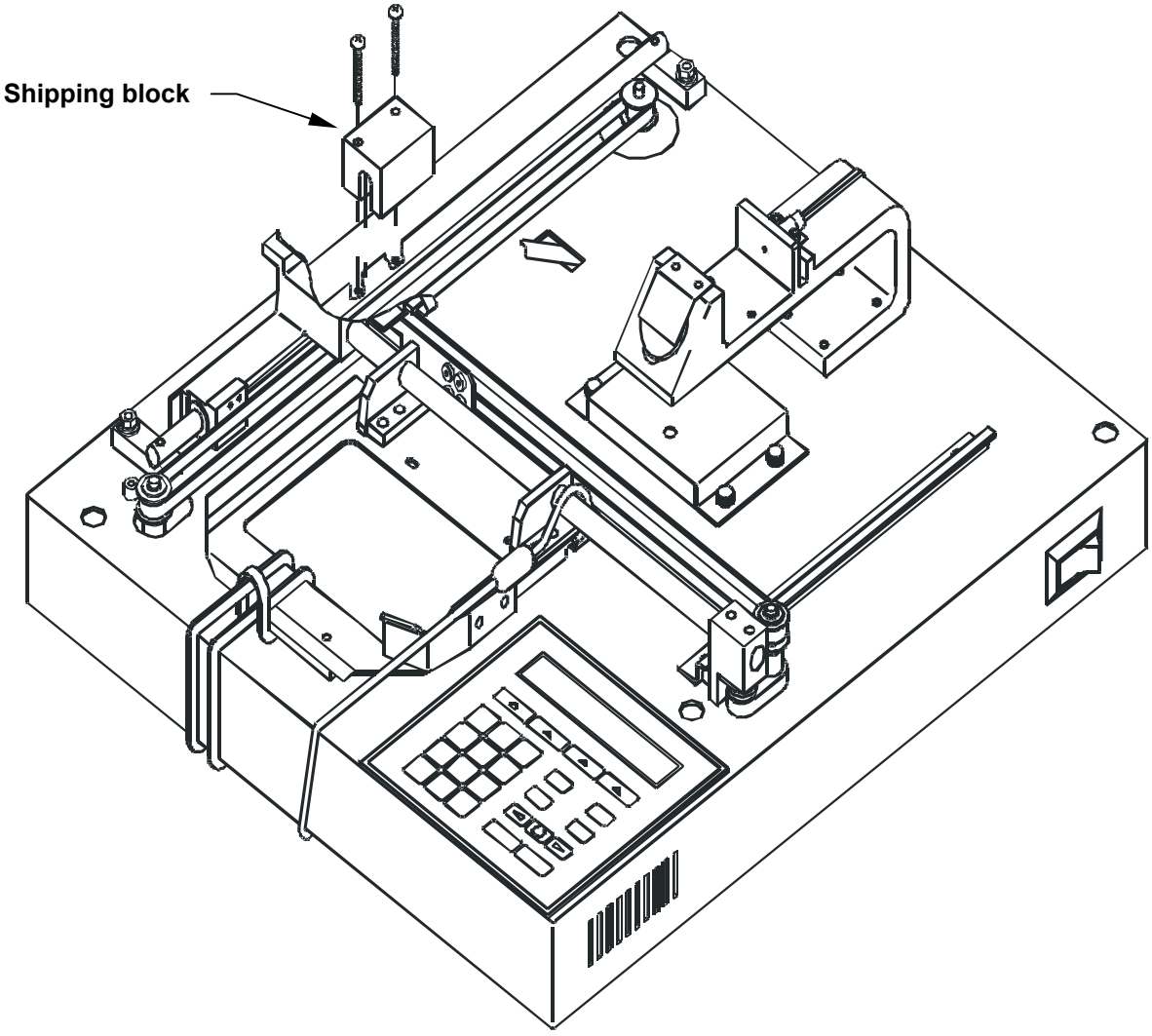


Figure 4: Removing the shipping block

8. Replace the top cover. Turn the instrument upside down and replace the mounting screws.
9. Remove the shipping straps from the top of the carrier. Save these for repackaging/shipping.

Setting Up the ELx800

Operating Environment

The ELx800 is designed to operate optimally when installed on a level surface in an area where ambient temperatures remain between 18°C (64.4°F) and 40°C (104°F). The reader is sensitive to extreme environmental conditions, and these conditions should be avoided:

- **Excessive humidity:** Condensation directly on the sensitive electronic circuitry can cause the instrument to fail internal self-checks.
- **Excessive ambient light:** Bright sunlight or strong incandescent light can reduce the linear performance range of the instrument.
- **Dust:** Optical density readings may be affected by extraneous particles (such as dust) in the microplate wells. A clean work area is necessary to ensure accurate readings.

Electrical Connections

Caution! Power Supply. Only use the specified power supply to ensure proper operation of the unit. The ELx800 has a universal 24-VDC, 2-Amp power supply that functions from 100 to 240 V~ (± 10.0%) @ 50 to 60 Hz without external switching.

Never use a two-prong plug adapter to connect primary power to the ELx800 power supply. Use of a two-prong adapter disconnects the utility ground, creating a severe shock hazard. Always connect the supply power cord directly to a three-prong receptacle with a functional ground.

1. Connect the power cord to the external power supply.
2. Plug the rounded end of the power supply line cord into the power supply jack on the rear of the instrument.
3. Tighten the knurled nut on the power supply outlet to ensure that the plug does not pull out.
4. Plug the 3-prong end of the power cord into an appropriate power receptacle.

Warning! Power Rating. The ELx800 power supply must be connected to a power receptacle that provides voltage and current within the specified rating for the system. Use of an incompatible power receptacle may produce electrical shock and fire hazards.

Power-Up and System Test

After you have installed the ELx800 and connected the power supply, turn on the instrument to run a system test. The on/off switch is located on the lower right side of the base.

The **System Test** begins with a check of the stepper motors and the analog power supplies, to ensure that they have a proper input voltage level. The data flash checksum, motor axis, and analog offset are then verified. The photodetector's dark current, noise, and gain are checked to ensure they fall within specific pass/fail criteria.

If an error is detected, the reader will "chirp" and display an error code. See **Chapter 6** for a list of error codes. If no errors are detected, the reader will briefly display **`SYSTEM TEST PASS`**.

The power-up system test does not produce a printed results report. To run the test manually and obtain a printout of the system test values, start at the Main Menu and press **`UTIL`** ➔ **`TESTS`** ➔ **`SYSTEM`**. See **System Test** and **Checksum Test** in **Chapter 4**, or **Connecting a Printer to the ELx800** on page 22 for more information.

ELx800 Main Menu

Following successful power-up of the ELx800, the Main Menu appears:

```
R E A D Y      0 1 : 2 2 P M      0 1 / 2 3 / 0 4

R E A D      D E F I N E   R E P O R T   U T I L
```

- The Main Menu permits access to all onboard functions. See **Main Menu** in **Chapter 3** for more information, including a diagram showing the flow of functionality (**Figure 14**).
- The ELx800 front panel contains four circular buttons, referred to in this manual as **"SOFT KEYS."** One **SOFT KEY** is positioned directly below each selectable option in the display. To select a menu option, simply press its corresponding **SOFT KEY**. See **ELx800 Front Panel** in **Chapter 3** for additional instructions.

Configuring Global Default Options

The ELx800 contains several global configurable options, such as date and time, report output, and plate reading preferences. These options are accessed via the Select Utility Option menu (**Figure 5** below), and include:

- **SETUP:** Set the current date and time, as well as the date and time formats.
- **OUTPUT:** Specify where plate data should be sent, to a printer, a computer, or to both. Additional options include report format (Column and/or Matrix), and whether or not to print standard curves.
- **READ:** Enable or disable read-time prompting for Plate ID, Sample ID, and Sample Count. Specify whether or not to read in Rapid mode.

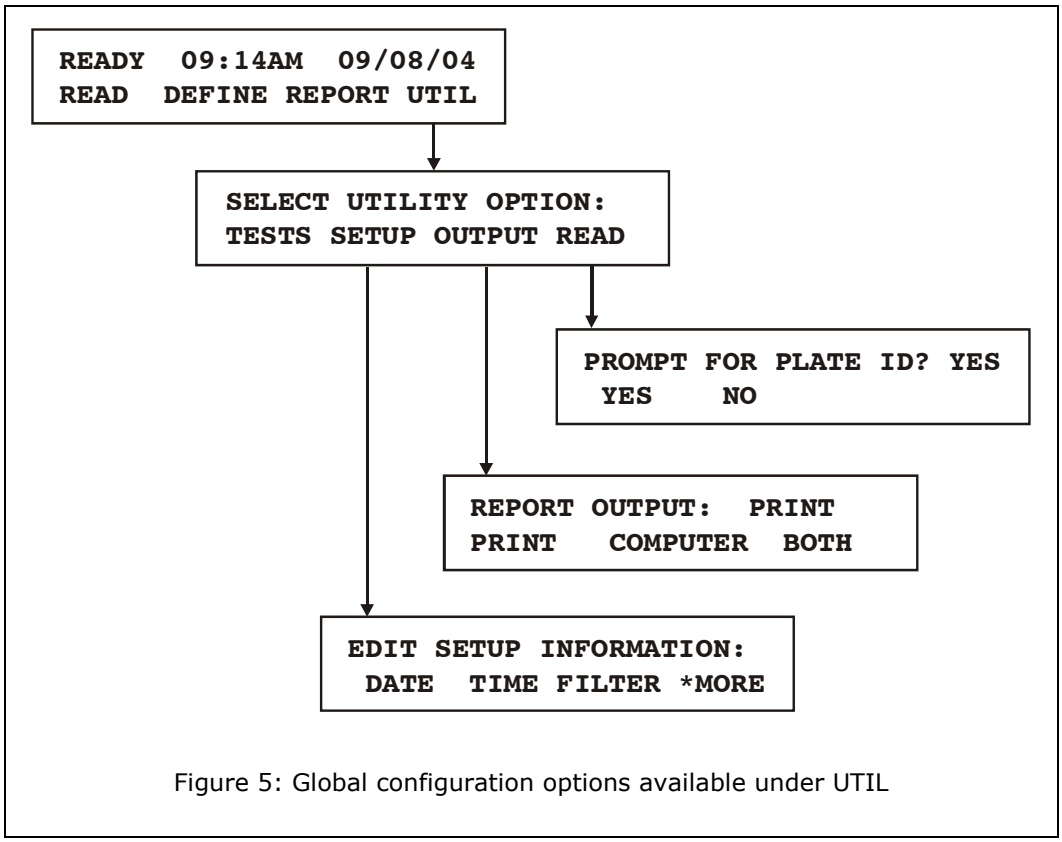


Figure 5: Global configuration options available under UTIL

❖ **Note:** Refer to **Using the Utility Options** in **Chapter 3** for more specific information on setting up the ELx800.

Connecting a Printer to the ELx800

Connect the printer to the ELx800 *only* if you are running the instrument in standalone mode. If you are using Bio-Tek's KCjunior™ or KC4™ software, skip this step, and go to page 24, **Setting Up the Serial Port for Communications**.

The ELx800 has a parallel port (LPT1) to allow connection to Epson-compatible printers or HP Deskjet™ printers. (See **Specifying Data Output and Reporting Options** in **Chapter 3** for more information.) The port is illustrated in **Figure 6**. The parallel port requires a 25-pin D-sub connector. A parallel cable (PN 71072) designed to connect the reader to a printer is provided with the ELx800.

To attach a printer to the ELx800:

1. Turn the reader off.
2. Place the printer in a location adjacent to the ELx800.
3. Attach one end of the cable to the parallel port on the printer.
4. Attach the other end of the cable to the parallel port on the ELx800.
5. Tighten the securing screws on both ends of the cable.
6. Turn on the reader, and then turn on the printer.

To avoid system instability, be sure to connect the printer to the reader *before* powering up the reader.

❖ **Note:** For the latest list of compatible printers, consult the Bio-Tek Web site (www.biotek.com), or call Bio-Tek Instruments' Technical Assistance Center (refer to **Chapter 1** for contact information).

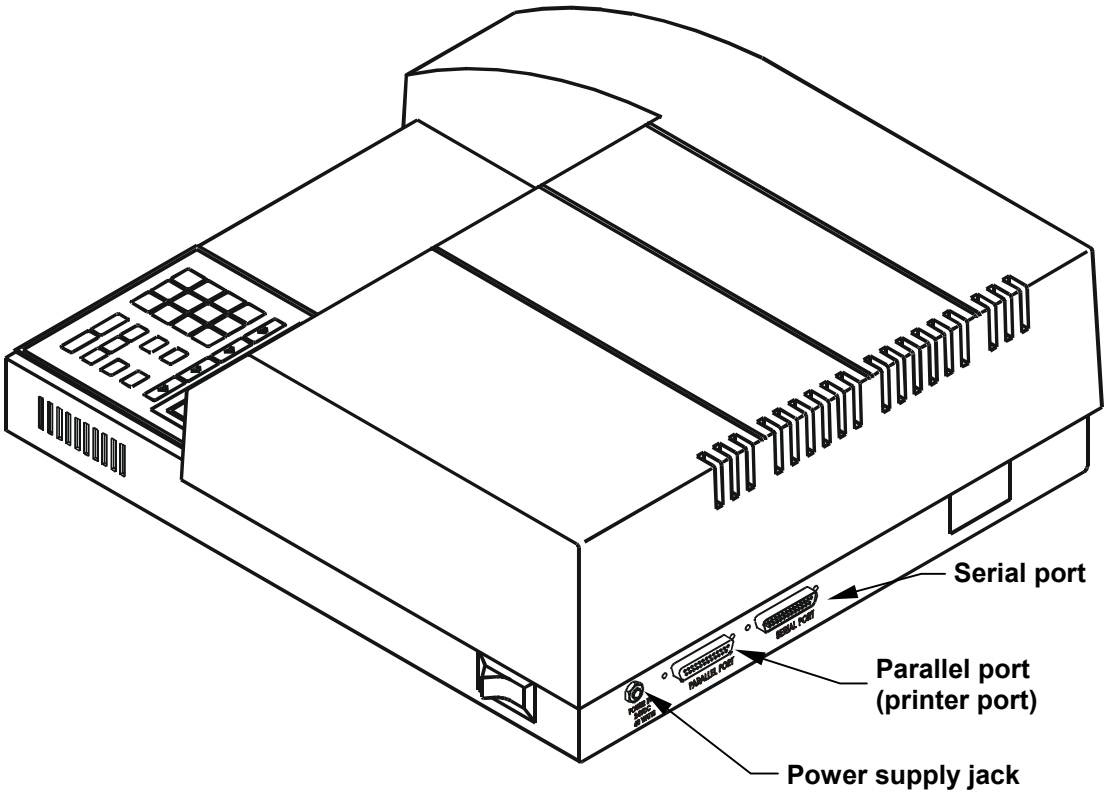


Figure 6: Connectors for printer (parallel), computer (serial), and power supply

Setting Up the Serial Port for Communications With Other Devices

Before serial communication can be initiated between the ELx800 and another device (such as a host PC running Bio-Tek's KCjunior™ or KC4™ software), the communication parameters must match between the devices.

The ELx800 has a 25-pin serial (RS-232) port located on the rear panel of the instrument. The serial port allows the reader to communicate with a computer, using standard communications software and/or RS-232 protocols.

The serial port also allows field upgrades of the ELx800 software.

> ❖ **Appendix A** contains information on required protocols for computer control of the reader.

Attaching the Cable

1. Power down the computer and the ELx800.
2. Connect the appropriate serial cable to both machines. The serial port on the reader is a DTE configuration with a 25-pin (pin-male) D-sub connector.
3. Power up the reader and the computer.
4. Ensure that the ELx800 and the computer are operating with the same communications settings.

Setting Communication Parameters

The reader's default communication parameters are:

- 9600 Baud Rate
- 8 Data Bits
- 2 Stop Bits
- No Parity

> ❖ The baud rate can be changed to 1200 or 2400 bps, if necessary. The Data Bits, Stop Bits, and Parity settings ***cannot*** be changed.

To change the baud rate from the instrument keypad:

1. At the Main Menu, press **UTIL**.

```
READY     12:45PM     01/23/04
READ    DEFINE  REPORT  UTIL
```

2. At the **SELECT UTILITY OPTION** screen, press **SETUP**.

```
SELECT UTILITY OPTION:
TESTS  SETUP  OUTPUT  READ
```

3. At the **EDIT SETUP INFORMATION** screen, press ***MORE**, and then **RS232** to continue.

```
EDIT  SETUP  INFORMATION
  DATE    TIME  FILTER  *MORE
```

```
EDIT  SETUP  INFORMATION?
RS232      CALPLATE      *MORE
```

4. The **SELECT BAUD RATE** screen will appear, showing the currently defined Baud Rate:

```
SELECT  BAUD  RATE:  9600
  1200    2400    9600    VIEW
```

- Select the desired baud rate.
- Select **VIEW**, if you wish, to see the reader's other communication settings.

```
RS232  SETTING:  NO  PARITY
2  STOP-BITS    8  DATA-BITS
```

To change the baud rate (or other communications settings) in KC4™ or KCjunior™, refer to their respective user guides, or to **Appendix A, Computer Control**.

Installing Additional Filters

Installed in the internal, five-position filter wheel are the filters that come standard with the ELx800 (standard models have 405, 450, 490, 630 nm filters; the UV model's filter set is 405, 450, 490, 630 and 340 nm).

Important! Keep track of all filter locations. The physical location of the filters must match the filter locations mapped in the reader's software filter table. The filter wheel must have no empty locations; all locations must be filled with either a filter or a blank plug. Install all filters with the arrow denoting the light direction pointing downward.

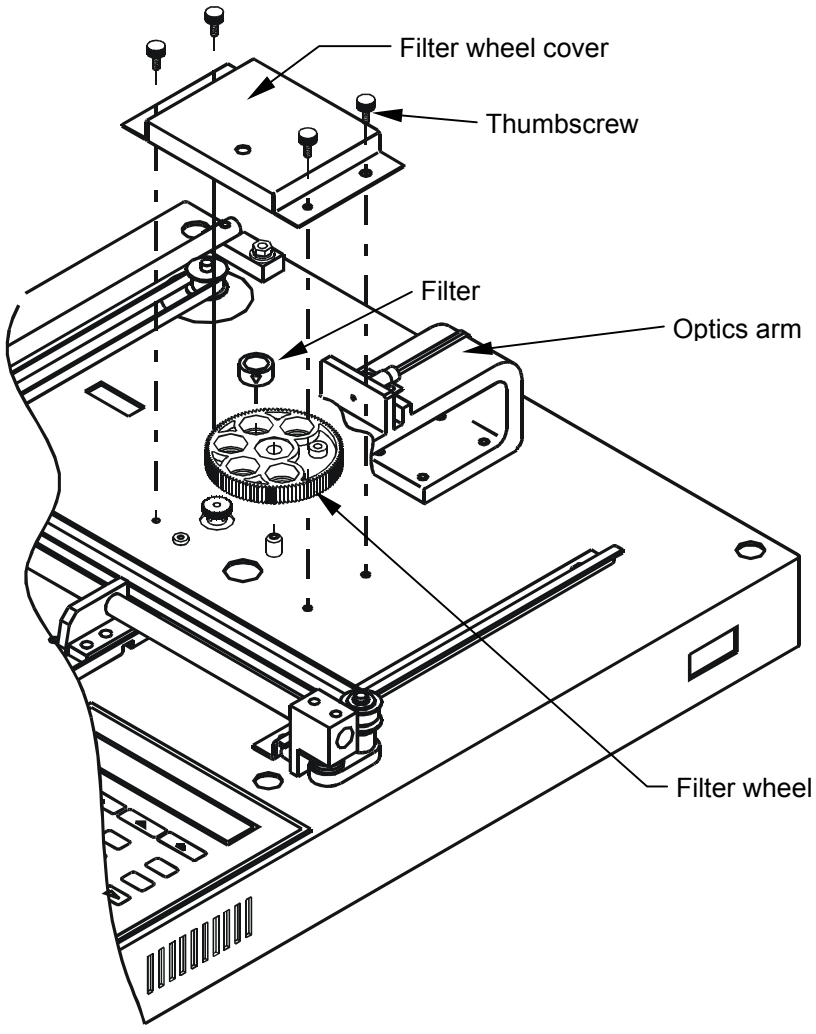


Figure 7: Filter wheel assembly

If you wish to install additional filters, or change the filter locations:

1. Turn off the unit and disconnect the power cord.
2. Tape down the carrier in the home (full out) position to prevent movement while the instrument is being handled.
3. Carefully turn the instrument upside down on a level surface.
4. Using a slotted screwdriver, remove the four screws from the bottom of the instrument (see **Figure 3**).
5. Hold on to both the instrument base and the top case to prevent the detachment of the case from the body of the instrument.
6. Carefully turn the instrument right side up and remove the top cover.

   With the cover off, the instrument's mechanics and optics are exposed. The filter wheel is housed within the small black box (filter wheel cover) directly under the end of the optics arm (**Figure 7**).

7. Remove the four thumbscrews around the perimeter of the cover. The filter wheel sits on a pin and can be lifted off.
8. Remove the filters by turning the wheel upside down over a cloth. The filters, which are labeled with the wavelength and light direction arrow, should easily slide out.
9. When replacing the filters in the wheel, handle them from the edges. Do not touch the glass portion of the filter. Clean any filters that appear dirty with lens paper and isopropyl alcohol.
10. Once the filters are installed in the filter wheel, place the wheel back on the pin in the base of the instrument, making sure the filter wheel is sitting flat and that it meshes with the filter wheel drive gear.
11. Install the filter wheel cover with the four thumbscrews.

❖ **Note:** The cover can only be installed one way.

12. Place the top cover on the base, and carefully turn the instrument upside down.
13. Reinstall the 4 screws with washers to hold the top cover in place.
14. Connect the power supply and cable to the rear of the instrument.

Important! Store unused filters in a cool, dry place away from direct sunlight. The filters can be wrapped in a piece of lens paper to protect them from scratches and dust accumulation.

Checking the Reader's Filter Table Setting

After installing new filters, ensure that the ELx800's filter table (the reader's software reference for filter locations) matches the physical location of the filters.

To check or change the software filter table:

1 Power up the reader. At the Main Menu Screen, press **UTIL** to display the **SELECT UTILITY OPTION** menu.

```
READY       9:45AM     01/31/03
READ    DEFINE   REPORT   UTIL
```

```
SELECT UTILITY OPTION:
TESTS  SETUP  OUTPUT  READ
```

2 From the **SELECT UTILITY OPTION** menu, press **SETUP**. The Edit Setup Information screen appears on the display.

```
EDIT SETUP INFORMATION:
 DATE    TIME   FILTER  *MORE
```

3 From this menu, press **FILTER**.

4 The wavelength for Filter #1 will be displayed. To advance to Filter 2, press **ENTER**.

5 To change the filter wavelength number, use the **NUMERIC** keypad to enter a number at the cursor location. The cursor will automatically advance to the next editable field. Press **ENTER** to save the entry and move to the next filter on the filter table.

```
E N T E R

F I L T E R # 1   W A V E L E N G T H : 4 0 5
```

When the last filter has been entered, the software exits the filter routine, and displays the following screen:

```
E D I T   S E T U P   I N F O R M A T I O N :

  D A T E     T I M E   F I L T E R   * M O R E
```

6 Press the Main Menu key to return to the main menu.

Repackaging and Shipping the ELx800

IMPORTANT! Failure to properly repackage the reader increases the likelihood of damage to the instrument during shipping. The shipping system was designed to stabilize the reader's mechanical mechanisms, which would otherwise be free to move around during shipping.

If you need to ship the ELx800 Reader to Bio-Tek for service or repair, be sure to use the original packing. Other forms of commercially available packing are not recommended and can **void the warranty**.

If the original packing materials have been damaged or lost, contact Bio-Tek for replacement packing (see **Technical Assistance** in **Chapter 1** for contact information).

Warning! If the reader has been exposed to potentially hazardous material, decontaminate it to minimize the risk to all who come in contact with the reader during shipping, handling, and servicing.

Decontamination prior to shipping is required by U.S. Department of Transportation regulations.

Before Repackaging the Instrument

1. **Decontaminate the reader before repackaging it**. (See **Chapter 5, Maintenance and Decontamination**, for the Decontamination procedure.)
2. Once the reader is clean, follow the instructions on the next few pages to repackage the instrument.

Repackaging the ELx800 and Its Accessories

Refer to **Figures 8** through **13** when repackaging the ELx800.

1. Move the carrier to the home position.
2. Turn off the unit and unplug the power supply.
3. Wrap the shipping straps around the carrier as shown in **Figure 8**.

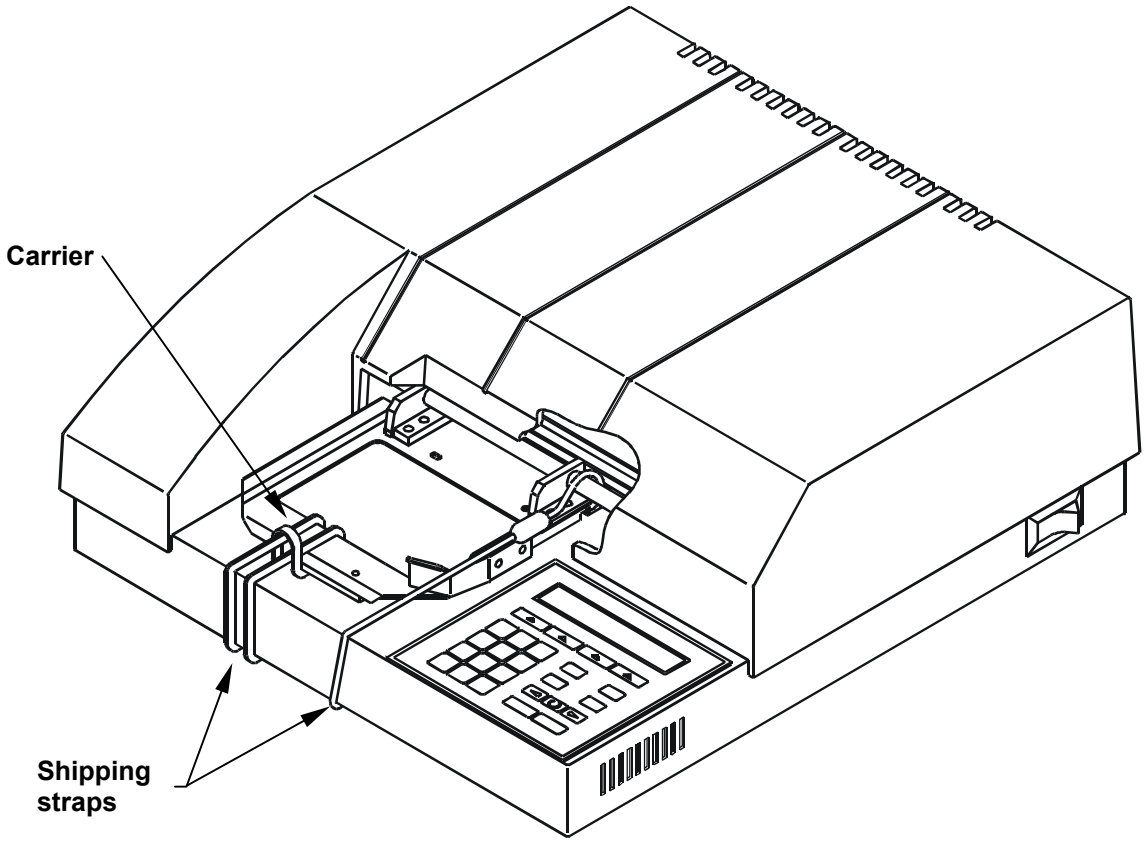


Figure 8: Securing the carrier

4. While holding the carrier, carefully turn the instrument over and hook the shipping straps around the shaft (**Figure 9**).
5. Remove the four top cover mounting screws.
6. Lift the instrument off the cover and turn it over.

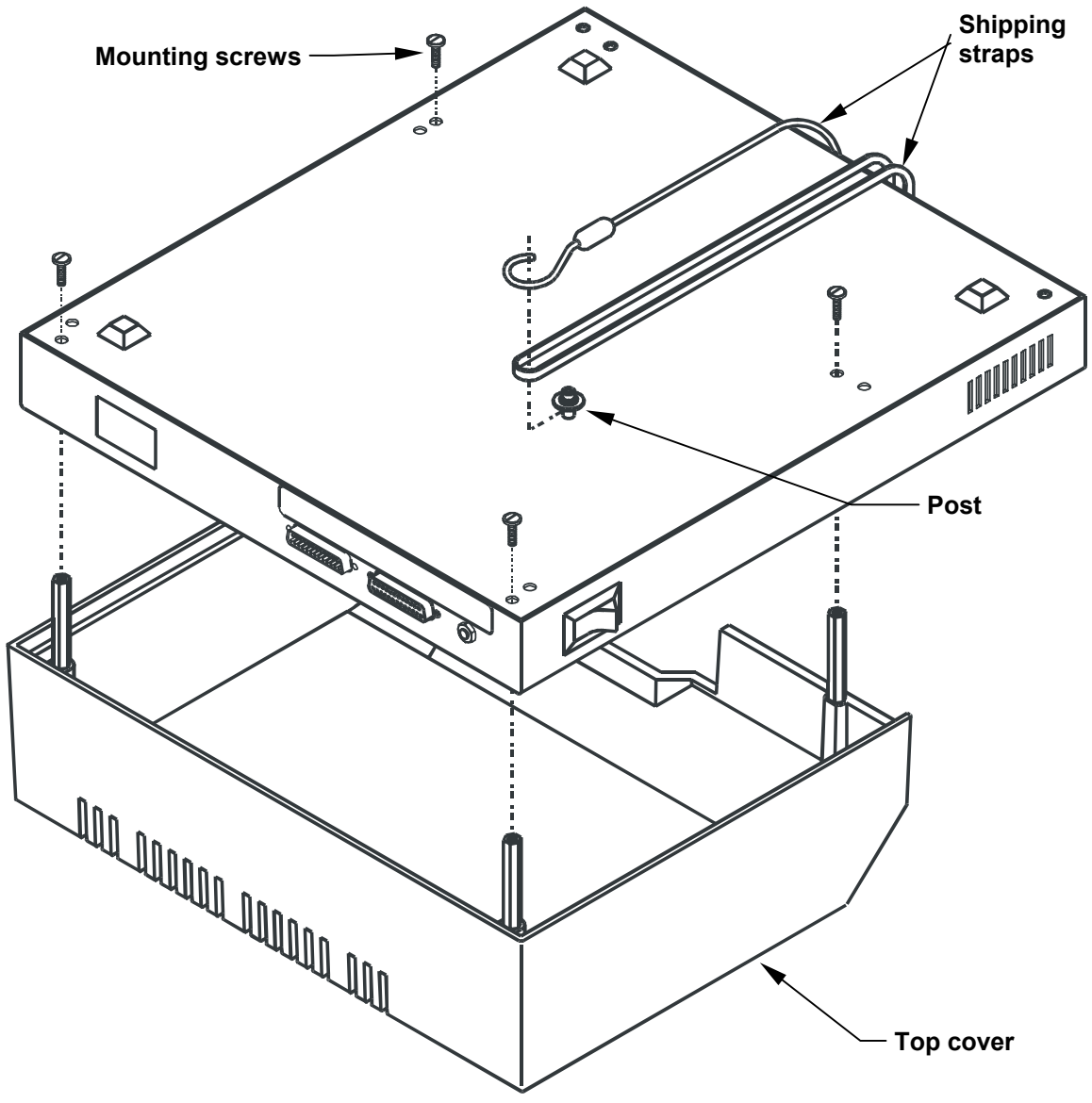


Figure 9: Hooking the shipping straps around the shaft

7. Install the shipping block. Place the groove in the shipping block over the shaft and screw it down (**Figure 10**).

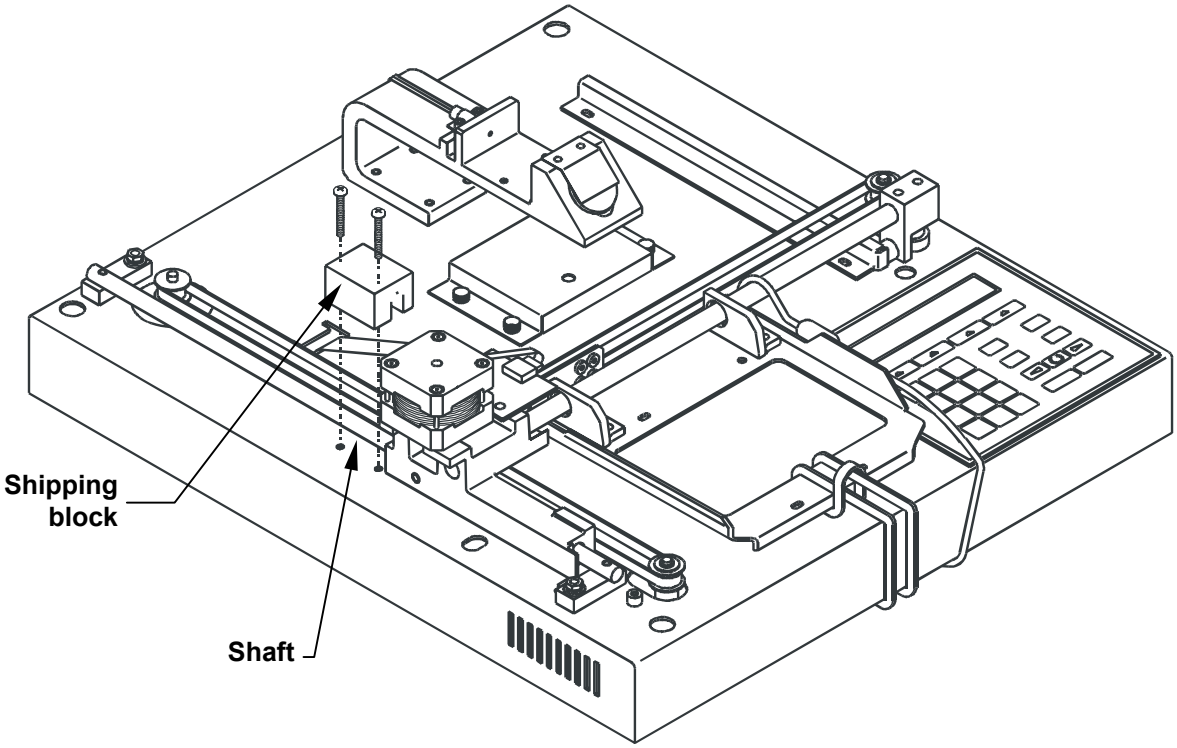


Figure 10: Installing the shipping block

8. Turn the instrument over and install the top cover that was removed in step 6 (**Figure 11**).

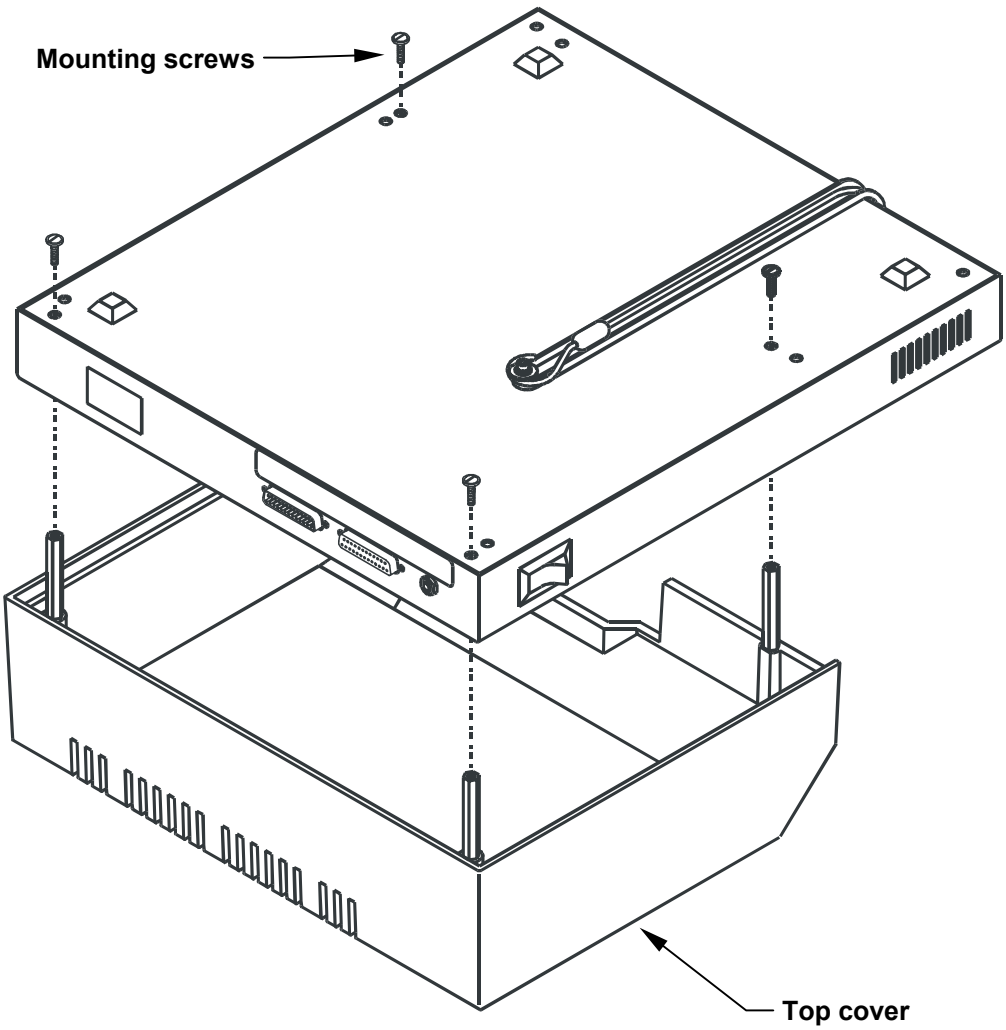


Figure 11: Reinstalling the top cover

9. Turn the instrument right side up, and put the end caps on the unit (**Figure 12**).

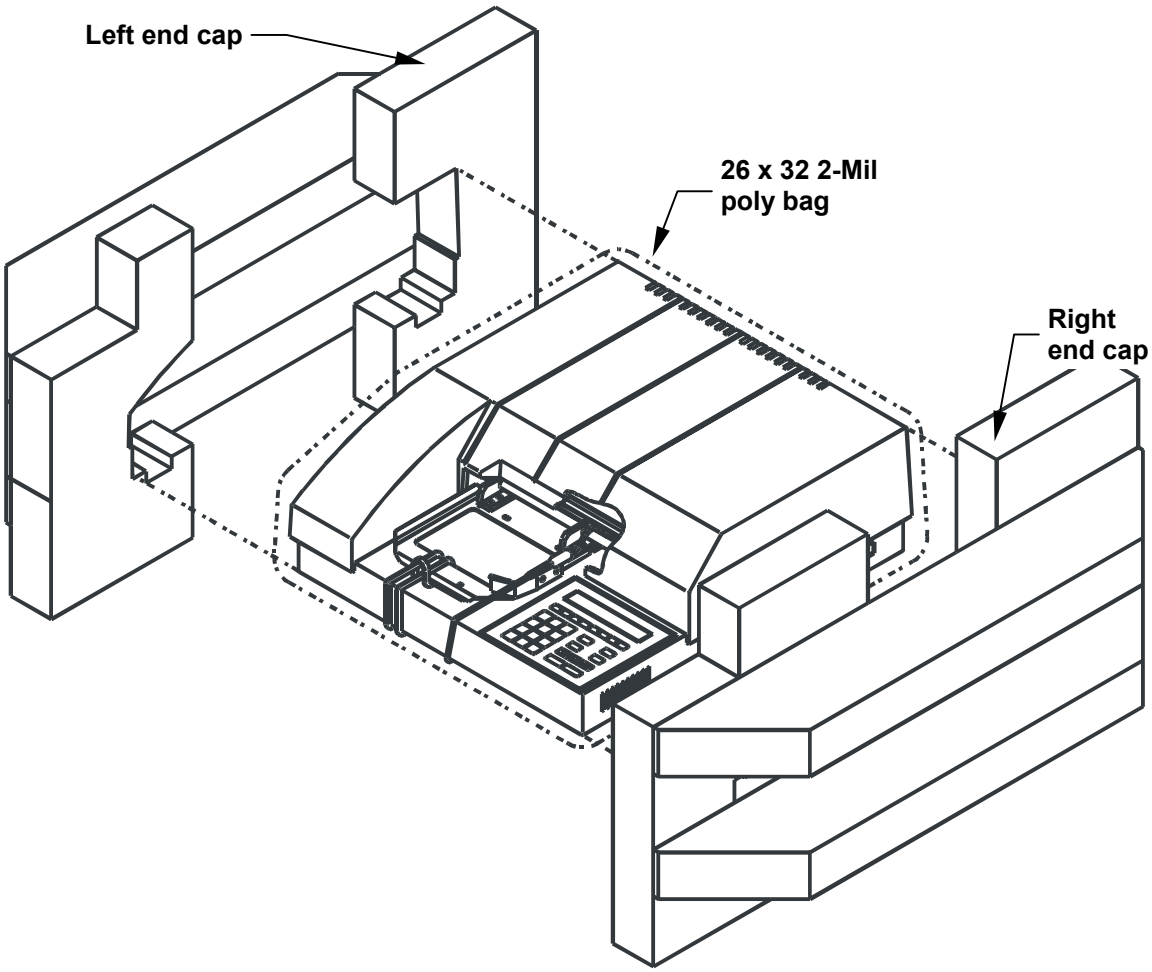


Figure 12: Reattaching the end caps

10. Place the unit with the end caps into the box (**Figure 13**).

11. Place the shelf into the notched-out area in the end cap, and the power supply into an 8″ x 11 ½″ bubble bag. Place the power supply on the shelf. (**Figure 13**).

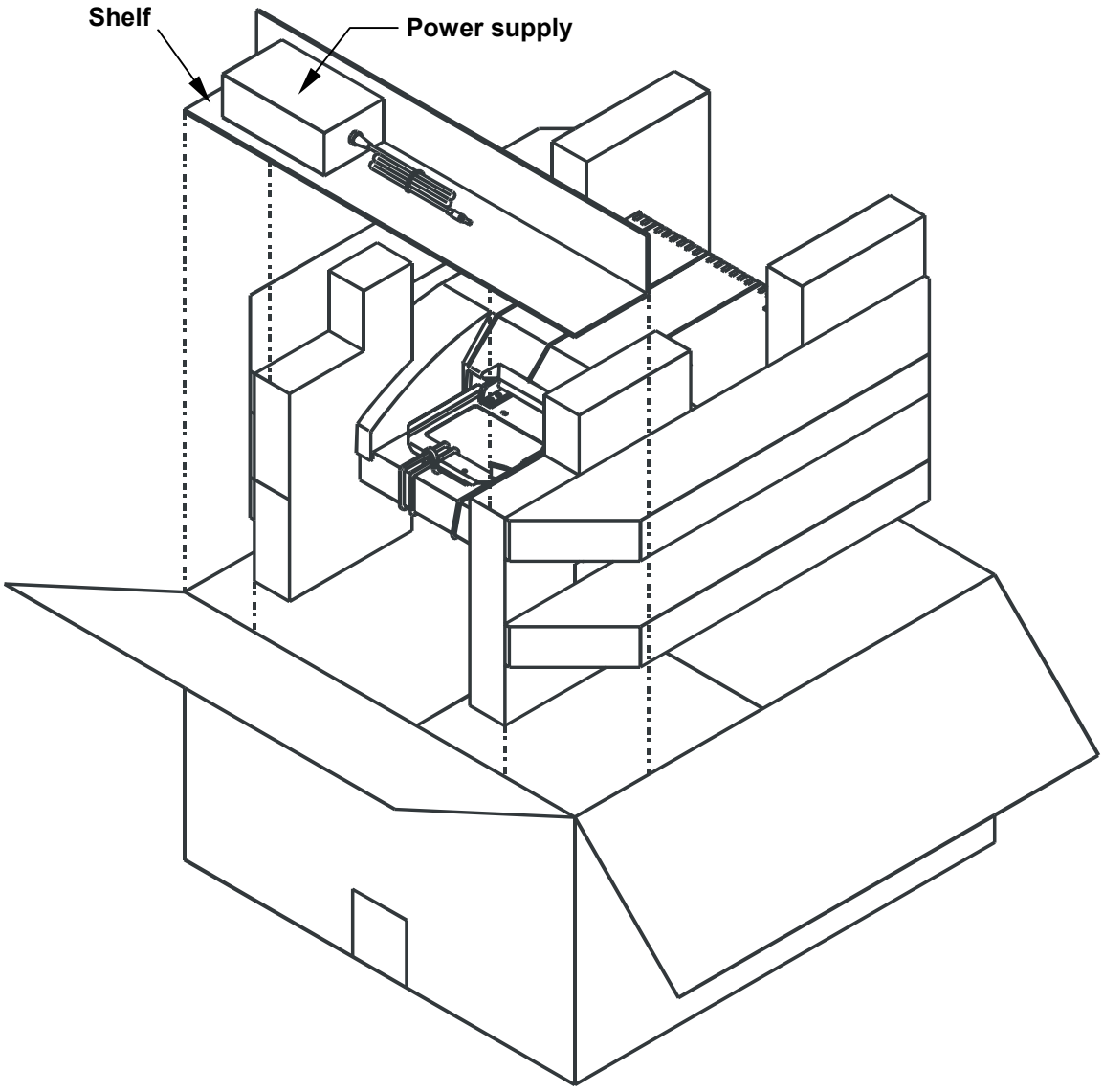


Figure 13: Placing the unit in the box

Preparing the Shipping Container

1. Obtain a Return Materials Authorization (RMA) number from Bio-Tek's Technical Assistance Center through Bio-Tek's Web site, fax, or e-mail address listed in **Chapter 1**.
2. When obtaining the RMA, explain whether the reader requires calibration, cleaning, periodic maintenance, warranty work, and/or repair. Make a note of any error messages displayed and their frequency.
3. Provide Bio-Tek with the name and contact information of a person who may be contacted if questions arise.
4. Close the box and tape it shut.
5. Write "RMA" and the RMA number in large, clear letters on the outside of the shipping container, and ship the instrument to the Bio-Tek address provided in the **Technical Support** section of **Chapter 1**.

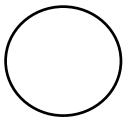
Chapter 3

Operation

This chapter includes instructions for operating the ELx800 and its software.

ELx800 Front Panel

Figure 14: Keyboard

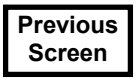


The keypad has four **SOFT KEYS,** one below each selectable menu option. Press a **SOFT KEY** to make a selection. For example, from the Main Menu, press the leftmost **SOFT KEY** to select **READ**, the rightmost to select **UTIL**.

Exit the current screen and return to the **Main Menu.** Pressing Main Menu while defining or modifying an assay automatically saves the current settings.

Cycle through available options within a screen. For example, press **Options** within the **Select Assay Number** screen to cycle through the names of the onboard assays.

Pressing **ENTER** generally saves the current screen settings and advances to the next screen in a series.



Pressing **Previous Screen** generally saves the current screen settings and returns control to the screen most previously viewed.

Press **CLEAR** to reset a numeric value to 0, or to clear all characters when editing an assay name. **Tip:** Press **Shift + Clear** at the **Map Generation** screen to "clear" a previously defined manual map.

Move the cursor to the left in data-entry screens.

Move the cursor to the right in data-entry screens.

Initiate a plate read.

Halt the read currently in progress.

7G

Press a number to enter it in a data-entry screen.

Shift + 7G

Press **Shift + a number** (for example, **Shift +** 7G) to enter the letter, then press **Options** to advance through the alphabet.

Overview

IMPORTANT! Do not turn on the instrument until the carrier shipping block has been removed.

The ELx800 features a 25-pad keypad and a 2-line x 24-character LCD display, allowing you to access the reader's program menus and print test results. The reader's bidirectional serial port allows computer control of the instrument, and provides the means for downloading additional assay definition files to the instrument. This chapter describes the operation of the ***open*** (configurable) assays onboard the ELx800.

Recommendations for Achieving Optimum Performance

Microplates should be perfectly clean and free of dust or bottom scratches. Use new microplates from sealed packages. Do not allow dust to settle on the surface of the solution; use microplate covers when not reading the plate. Filter solutions to remove particulates that could cause erroneous readings.

Although the readers support standard flat, U-bottom, and V-bottom microplates, optimum performance is achieved with optically clear, flat-bottomed wells.

Non-uniformity in the optical density of the well bottoms can cause loss of accuracy, especially with U- and V-bottom polyvinyl microplates. Check for this by reading an empty microplate. Dual-wavelength readings can eliminate this problem, or bring the variation in density readings to within acceptable limits for most measurements.

Inaccuracy in pipetting has a large effect on measurements, especially if smaller volumes of liquid are used. For best results, use at least 100 µl per well in a 96-well plate and 25 µl in a 384-well plate.

Dispensing solution into 384-well plates often traps air bubbles in the wells, which may result in inaccurate readings. A dual-wavelength reading method usually eliminates these inaccuracies; however, for best results, remove the air bubbles by degassing the plate in a vacuum chamber before reading.

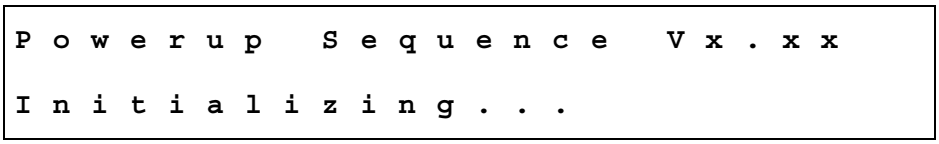
The inclination of the meniscus can reduce reading accuracy in some solutions, especially with small volumes. Agitate the microplate before reading to help bring this problem within acceptable limits. Use Tween® 20, if possible (or some other wetting agent) to normalize the meniscus. Some solutions develop menisci over a period of several minutes. This effect varies with the brand of microplate and the solution composition. As the center of the meniscus drops and shortens the light path, the density readings change.

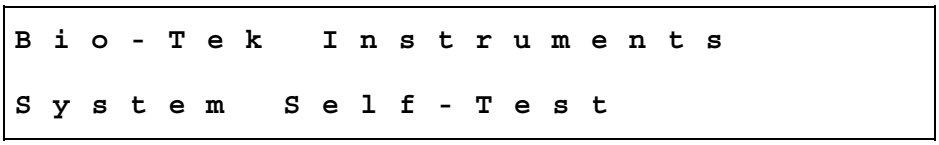
System Startup

To turn on the ELx800, press the on/off switch on the right side of the reader's base. The ELx800 will perform a System Test, displaying the screens shown below until initialization is complete. During this period, all keys are inactive.

If the instrument fails the System Test, a chirp will sound, and an error code will display.

- Refer to **System Self-Test** and **Checksum Test** in **Chapter 4** for more information.
- Refer to **Chapter 6, Troubleshooting and Error Codes** to interpret error codes, and **Chapter 1, Introduction** for information on contacting Bio-Tek Instruments' Technical Assistance Center (TAC).





Main Menu

Following successful power-up of the ELx800, the Main Menu appears:

```
R E A D Y        9 : 4 5 A M      0 1 / 3 1 / 0 3

R E A D     D E F I N E   R E P O R T   U T I L
```

The Main Menu permits access to all reader functions (**Figure 15**):

- **READ** option (or, press the key labeled READ on the keyboard) for plate-reading prompts. You will be asked to select from a list of preprogrammed assays.
- **DEFINE** option: Allows the creation of a reading and data reduction protocol. You will be prompted to select an assay from an assay definition list.
- **REPORT** option: Allows printing of results and protocol descriptions. You will be prompted for the name of a previously run assay with valid data.
- **UTIL** option: You will be prompted to select options from the list of onboard utilities.

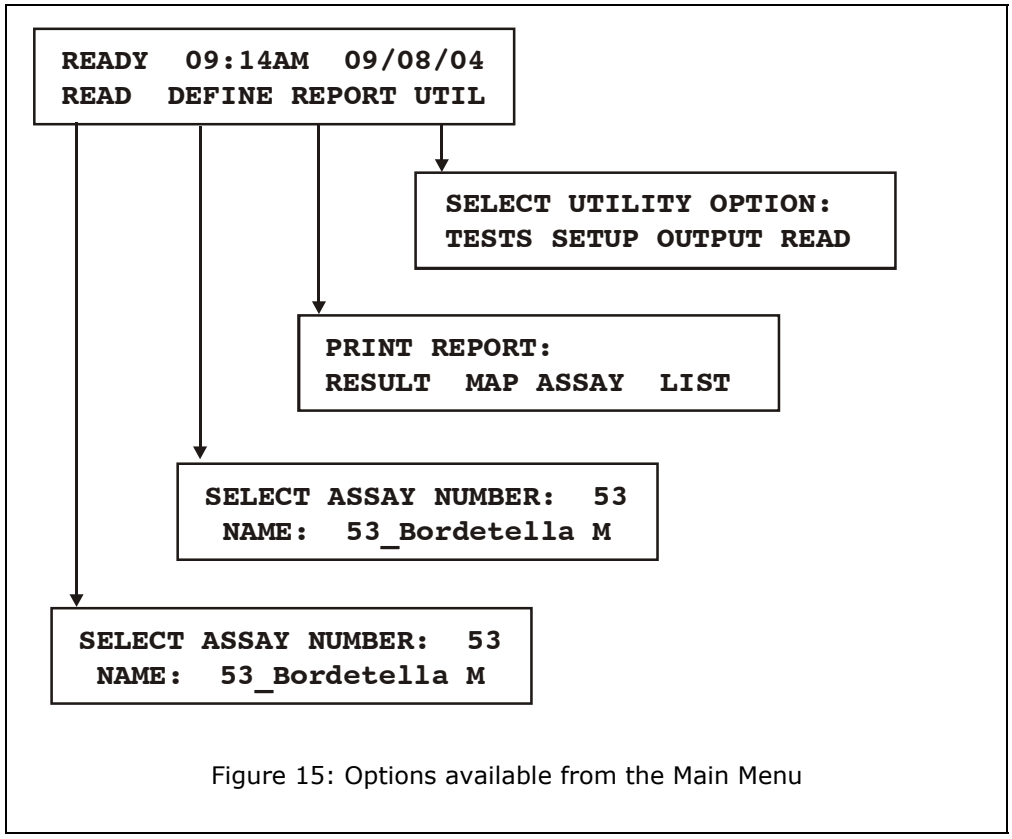


Figure 15: Options available from the Main Menu

Important: On some Universal Microplate Readers, **Assay 01** has been designed to allow for quick and simple programming. It appears as "_Quick Read" on the display. Most of the options available in Assays 2-55, and described in this section, are unavailable for programming within Quick Read. You can quickly access the Quick Read assay by selecting **READ** from the main menu.

The Quick Read assay default **DEFINE** settings are shown below, and cannot be edited, except where noted.

Method

- Single Wavelength 405 nm (editable)

Map

- 96-well plate geometry
- Blank on Air
- Automap
- Map starting location A1
- Samples only (no blanks, standards or controls)
- Sample count prompted at runtime (can be turned off in UTIL|READ options)

Define

The Main Menu option **DEFINE** allows you to define the data acquisition and reduction parameters for a new assay, or modify previously defined assays stored in memory.

1. Start at the Main Menu and select **DEFINE** to display the **SELECT ASSAY NUMBER** screen.

```
S E L E C T   A S S A Y   N U M B E R : 0 1

N A M E : H B S -A G 1
```

2. Select an assay to define or modify, and then press **ENTER**. See **Selecting an Assay to Define** on page 48 for detailed instructions. The **EDIT ASSAY NAME** screen will appear.

❖ If you are modifying/selecting a PANEL assay (#99), see page 85 for instructions.

```
N A M E : H B S -A G 1

    -     /          :                  S P A C E
```

3. (Optional) Edit the assay name, and then press **ENTER**. See **Editing the Assay Name** on page 48 for detailed instructions. The **DEFINE** menu will appear:

```
D E F I N E :

M E T H O D   M A P   F O R M U L A   C U R V E
```

The following options are available within the **DEFINE** menu:

- **METHOD:** Define the wavelength type (single or dual), wavelength(s), and plate geometry (page 49).
- **MAP:** Specify the plate layout, using blanks, controls, standards, and/or samples. Choose to map the plate manually, or let the software map it automatically (page 53).
- **FORMULA:** Define cutoff, transformation, and validation formulas. Create variables to be used within formulas (page 69).
- **CURVE:** Specify a curve fit type and x/y axis types (lin/log). Specify whether or not standard outliers can be edited, and then the method by which they will be edited. Enable or disable the extrapolation feature (page 81).

Programming Note

Assays with certain criteria may "lock up" during the "Calculating Results…" phase of data reduction (see **Beginning the Plate Read** on page 92). This can be corrected by modifying assay criteria as follows:

- Specify **at least one (01)** sample when mapping the plate, and/or
- Change **PROMPT SAMPLE COUNT?** to **YES**.
- Set **CURVE FIT** to **NONE** if you do not have a standard curve as part of the assay.

1 From the Main Menu, press **DEFINE** ➔ **MAP**, and continue to press **ENTER** until **ENTER NUMBER OF SAMPLES** is displayed. Enter at least one sample.

❖ The software will lock up if zero (00) samples are selected.

```
E N T E R   N U M B E R   O F
S A M P L E S :                    0 1
```

2 From the Main Menu, press **UTIL** ➔ **READ**, and continue to press **ENTER** until **PROMPT SAMPLE COUNT?** is displayed. Select **YES**.

```
P R O M P T   S A M P L E   C O U N T ?   Y E S
  Y E S         N O
```

3 From the Main Menu, press **DEFINE** ➔ **SELECT ASSAY NUMBER:** ➔ **ENTER**. At the **DEFINE:** menu, press **CURVE** to display **CURVE-FIT TYPE:** and select **NONE**.

```
C U R V E - F I T   T Y P E :   N O N E
  N O N E   L I N E A R   Q U A D     * M O R E
```

Selecting an Assay to Define

To select an assay to define or modify, start at the Main Menu and select **DEFINE** to display the **SELECT ASSAY NUMBER** screen.

```
S E L E C T   A S S A Y   N U M B E R : 0 1

N A M E : H B S -A G 1
```

1. Use the **numeric** keys to enter the number of any predefined Assay Definition Files stored in the reader's memory, or the **Options** key to advance one assay at a time. The cursor is positioned at the first editable field, and advances automatically. The numeric range depends on the number of assays stored in the reader's memory.

❖ The ELx800 has 55 "open" assays available; the EL800 has only 10.

2. Press **ENTER** to advance to the **EDIT ASSAY NAME** screen. You may change the default assay name to a more descriptive one (see **Editing the Assay Name** below):
   - **CLEAR:** Clears the reader's display.
   - **MAIN MENU:** Returns the display to the Main Menu screen.
   - **PREVIOUS SCREEN:** Returns the display to the previous screen.
   - **ENTER:** Saves the current settings and advances to the next screen.

Editing the Assay Name

Use the **EDIT NAME** screen to edit the name currently assigned to the assay. The assay name can contain up to 16 alphanumeric characters.

```
N A M E :       H B S - A G 1

  -             /           :          S P A C E
```

- The cursor is positioned at the first editable field (e.g., under "H"). Use the **alpha** and **numeric** keys to change the assay name.
- Use the **Options** key to sequentially advance the character positioned above the cursor. The characters will cycle through the alphabet (A-Z), with a space following Z.
- Use the **left** and **right arrow** keys to move the cursor to the previous or next editable field. The cursor will wrap around the edit field.
- Use the **CLEAR** key to remove the assay name from the display.
- Use **SOFT KEYS 1, 2, 3,** and **4** to select a dash, forward slash, colon, or space for inclusion in the assay name.

Define (Method, Map, Formula and Curve)

The **DEFINE** screen allows you to edit the **Method, Map, Formula,** or **Curve Fit** parameters for the currently selected assay.

```
D E F I N E :

M E T H O D   M A P   F O R M U L A   C U R V E
```

Press the **SOFT KEY** beneath the displayed option to access the following functions:

- **METHOD:** Specify the wavelength type, wavelength, filter(s), and plate geometry.
- **MAP:** Specify mapping information.
- **FORMULA:** Access the formula entry screens.
- **CURVE FIT:** Specify curve-fit options.

Defining METHOD

Defining a method includes selecting:

- Single or dual wavelength
- Filter(s)
- Plate geometry

The options appear on the display in the order that they were programmed in the assay. If the assay contains a closed variable (i.e., an element of the assay definition that you cannot access or modify), the entry screen is skipped.

Single or Dual Wavelength

```
W A V E L E N G T H :   D U A L

S I N G L E   D U A L
```

1. Select **SINGLE** or **DUAL** wavelength.
2. Press **ENTER** to continue.

The **WAVELENGTH** selection screen allows you to select **SINGLE** or **DUAL** wavelength for the assay.

If **SINGLE** wavelength is chosen, the reader measures the optical density of each well with a single filter.

If **DUAL** wavelength is chosen, each well is read twice, each time with a different filter. The microplate is not removed from the reading chamber between the two measurements. The final reported optical density is the difference between the two readings (the delta OD). Dual-wavelength readings can significantly reduce optical interference caused by scratched or fingerprinted microplates, since the scratches or fingerprints reduce the amount of light on both wavelengths.

The currently selected wavelength appears on the top line of the display, and the available options appear on the bottom.

MEAS Selection

The **MEAS** selection screen allows you to select the filter(s) for the assay.

```
M E A S : 4 5 0   R E F : 6 3 0

4 0 5         4 5 0       4 9 0         6 3 0
```

1. Select the wavelength.
2. Use the right arrow key to move the cursor to **REF** and then select the Reference Filter.
3. Press **ENTER** to move to the next screen.

Plate Type

For 6- to 384-well standard plates, the plate types and sizes included in the software onboard the ELx800 are based on the brands listed below. For best measurement results, use these brands when operating the ELx800 via its onboard software.

Plate Type	Brand
6-well	Costar
12-well	Corning
24-well	Corning
48-well	Costar
96-well	Costar
384-well	Nunc square*
96 T	Terasaki*
72 T	Terasaki*
60 T	Terasaki*

*Used only on ELx800NB version

The **PLATE TYPE** selection screen allows you to select the geometry of the plate that will be used for the assay. Press ***MORE** to cycle through the available options.

❖ **Note:** The NB version of the reader includes choices for 384-well, or 72- and 96-well Terasaki plate formats. An adapter (P/N 7330531) is available from Bio-Tek if Terasaki plates are to be used with the NB reader.

```
P L A T E   T Y P E :   9 6   W E L L S

2 4          4 8          9 6          3 8 4
```

```
P L A T E   T Y P E :          9 6   W E L L S

      6          1 2          2 4       * M O R E
```

```
P L A T E   T Y P E :          9 6   W E L L S
   4 8         9 6       9 6 H       * M O R E
```

```
P L A T E   T Y P E :          9 6   W E L L S
  9 6 M                             * M O R E
```

Press ***MORE** to cycle through the available options.

- **6:** 6-well (2 x 3)
- **12:** 12-well (3 x 4)
- **24:** 24-well (4 x 6)
- **48:** 48-well (6 x 8)
- **96:** 96-well (8 x 12)
- **96H:** 96-well Hellma Quartz (8 x 12)
- **96M:** 96-well Metric (8 x 12, 9 mm well spacing)

Defining MAP

The **MAP GENERATION** screen allows you to edit or specify the following options in the assay:

- Automatic or manual map generation
- Mapping direction
- Replication direction
- Blank Map Selection
- Blanking constant
- Number of Blanks
- Location of Blanks
- Number of Standards
- Number of Standard Replicates
- Averaging of Standards
- Concentration and Location of Standards
- Number of Controls
- Control Type Definition
- Number of Control Replicates
- Control Location
- Number of Samples
- Number of Sample Replicates
- Sample Location

Important! The NB reader using 384-well geometry does not have access to map, formula, or curve options. The 384-well plate type is preset for the map to have 384 samples. This does not include any blanking, controls, or standards. (Selecting 6-, 12-, 24-, 48-, or 96-well geometries enables data reduction capabilities.)

MAP screens appear in the order that they were defined in the assay. If the assay has a closed variable, the screen for this variable is omitted.

```
D E F I N E :

M E T H O D   M A P   F O R M U L A   C U R V E
```

At the **DEFINE** options screen, select **MAP** to begin the plate map process.

Map Generation

"Map Generation" represents the method by which blanks, controls, standards, and/or samples are assigned to specific locations on the plate.

```
M A P   G E N E R A T I O N :   M A N U A L

A U T O     M A N U A L
```

- Select **AUTOMATIC PLATE MAP GENERATION** to instruct the software to automatically generate a plate map after the blanks, controls, standards, and/or samples have been defined.
- Select **MANUAL PLATE MAP GENERATION** to indicate that the well assignments will be performed manually (by the user) at Define and/or Read time.
- Press **ENTER** to save the selection and continue.

Use the **SHIFT-CLEAR** keys to clear any previously defined manual map.

Mapping Direction

This option allows you to specify how the blank, control, standard, or sample ***groups*** will be mapped on the plate. The well types can be listed in column format (down) or in row format (across). The currently selected Mapping Direction appears on the top line of the display, and the available options appear on the bottom.

```
MAPPING  DIRECTION: DOWN
DOWN     ACROSS
```

- Select **DOWN** to map down the column.
- Select **ACROSS** to map across the row.
- Press **ENTER** to save the selection and continue.

Replication Direction

This option allows you to specify how ***replicates*** are mapped on the plate. The currently selected Replication Direction appears on the top line of the display, and the available options appear on the bottom.

```
REP  DIRECTION: ACROSS
DOWN     ACROSS
```

- Select **DOWN** to map the replicates down the column, following the direction of the map listing.
- Select **ACROSS** to map the replicates across (in a paired format). As an example, two replicates can be placed in A1 and A2 wells. The third replicate would follow in B1. The next standard control, or sample, would follow in B2.
- Press **ENTER** to save the selection and continue.

Examples of mapping directions are shown on the next page.

Examples of Mapping Directions

Map Direction DOWN, Rep Direction DOWN:

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1	STD5	SMP									
B	STD1	STD5	SMP									
C	STD2	PC	SMP									
D	STD2	PC										
E	STD3	NC										
F	STD3	NC										
G	STD4	SMP										
H	STD4	SMP										

Map Direction ACROSS, Rep Direction ACROSS:

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1	STD1	STD2	STD2	STD3	STD3	STD4	STD4	STD5	STD5	PC	PC
B	NC	NC	SMP	SMP	SMP	SMP	SMP	SMP				
C												
D												
E												
F												
G												
H												

Map Direction DOWN, Rep Direction ACROSS:

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1	STD1										
B	STD2	STD2										
C	STD3	STD3										
D	STD4	STD4										
E	STD5	STD5										
F	PC	PC										
G	NC	NC										
H	SMP	SMP										

Map Direction ACROSS, Rep Direction DOWN:

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1	STD2	STD3	STD4	STD5	PC	NC	SMP	SMP			
B	STD1	STD2	STD3	STD4	STD5	PC	NC	SMP	SMP			
C												
D												
E												
F												
G												
H												

Start Mapping at Well Location

The Start Mapping at Well Location screen is only shown if automatic mapping is selected. This option allows you to enter the location of the well that will be the starting point for automatic mapping.

```
S T A R T   M A P P I N G

A T   W E L L   L O C A T I O N : A 0 1
```

- Use the **numeric** and **alpha** keys to enter a letter or number at the cursor location. For any well location, only the **alpha** keys are active for the first character and **numeric** for the second and third characters. The valid entry range is from A01 to the last well on the plate, depending on the plate type and the number of blanks, standards, controls, and/or samples defined in the assay.
- Press **ENTER** to save the well location and continue.

Blank Map

This option allows you to select which blanking method to apply to the assay.

The blanking options, **AIR**, **FULL** and **CONSTANT**; **ROW** and **COLUMN**; and **P-ACROSS** and **P-DOWN** are displayed on three screens.

```
B L A N K   M A P :          F U L L
  A I R       F U L L     C O N S T   * M O R E
```

```
B L A N K   M A P :          F U L L
  R O W     C O L U M N               * M O R E
```

```
B L A N K   M A P :          F U L L
    P - A C R O S S     P - D O W N   * M O R E
```

- Select the **BLANK MAP** type (see the descriptions on the next page).
- Press ***MORE** to cycle through the available options: **ROW** or **COLUMN**, and **P-ACROSS** or **P-DOWN**.
- Press **ENTER** to save the well location and continue.

Blank Map Definitions

- **AIR** performs an initial reading on "air" just prior to the plate read, and uses that value as the blank value. This value is subtracted from each well on the plate.
- **FULL** enables a single blank well or an average of blank wells to be subtracted from the whole plate.
- **CONST** (Constant) allows entry of a user-specified absorbance value. This value will be subtracted from each well on the plate.

❖ **Tip for using CONST:** Use a blank value from the first plate, or a blanking plate, to save space on subsequent assay plates.

- **ROW** enables a single blank well or an average of blank wells to be selected for each row. The maximum number of blanks is 48. The blank (or average) will be subtracted from each well in the row. Use manual mapping to position blanks, controls, standards, and samples.
- **COLUMN** enables a single blank well or an average of blank wells to be placed in each column. Since the maximum number of blanks is 12, and if all 12 columns are used, each column can have only one blank. Manual mapping is recommended in this case. Replicates follow in the same column as the first well of each sample, even if the ACROSS direction has been specified for replicates.
- **P-ACROSS** enables a blank in every even-numbered column to be subtracted from the well to the left of it in every odd column. Manual mapping is recommended to set up the appropriate map by placing the standards, controls, and samples in only the odd columns.
- **P-DOWN** enables a blank in the B, D, F and H rows to be subtracted from the well above in the A, C, E and G rows. Manual mapping is recommended to set up the appropriate map by placing the standards, controls, and samples in only the A, C, E, and G rows.

Constant Blank Value

This entry screen only appears when a **CONSTANT BLANK** map is selected. Enter the value to be subtracted from each well on the plate.

```
E N T E R

B L A N K I N G   C O N S T A N T :   1 . 2 0 0
```

- Use the **numeric** keys to enter the value. The range is 0.000 to 3.000. The cursor is positioned at the first editable field and advances automatically.
- Press **CLEAR** to clear the value on the display.
- Press **ENTER** to continue.

Number of Blanks

The **NUMBER OF BLANKS** field allows you to enter the number of blank wells in the assay. This entry screen is only displayed when Full, Column, or Row blank maps is selected. Any previously defined value is displayed.

```
E N T E R   N U M B E R   O F

B L A N K S :                    0 2
```

- Use the **numeric** keys to enter the number of blanks. The range is 0 to 48.
- Use the **CLEAR** key to clear the **NUMBER OF BLANKS** value from the display.
- Press **ENTER** to continue.

Blank Location

The **BLANK LOCATION** screen allows you to define where the blank well or wells occur on the microplate. This screen only appears if **manual mapping** was selected.

```
E N T E R   T H E   L O C A T I O N   O F
B L A N K #   1 :                    A 1 2
```

- Use the **numeric** and **alpha** keys to enter a Blank Location, based upon the plate geometry.
- Use the arrow keys to move the cursor to the next or previous editable field. The cursor is positioned beneath the first editable field.
- Press **ENTER** to continue.

Number of Standards

This option allows you to enter the number of standard ***groups*** that will be used in the assay. Any previously defined value will be displayed on the screen.

> ❖ **Note:** If the number of standards is altered, the number of replicates for the standard automatically reverts to 1.

```
E N T E R   N U M B E R   O F
S T A N D A R D S :                  0 2
```

- Use the **numeric** keys to enter the **NUMBER OF STANDARDS**. The valid range depends on the selected curve fit method. The maximum number of standards is 12. The minimum is 4 for 4-P fit, cubic, cubic spline, and logit-log; 3 for quadratic; and 2 for linear and point-to-point.
- Press **CLEAR** to clear the value on the display.
- Press **ENTER** to continue.

Number of Standard Replicates

This option allows you to enter the number of replicates per standard group in the assay. Any predefined value appears on the display.

```
E N T E R   N U M B E R   O F
S T A N D A R D   R E P L I C A T E S :     0 2
```

- Use the **numeric** keys to enter the **NUMBER OF STANDARD REPLICATES**. The range is 1 to 8 replicates. The software will verify that the number of replicates, multiplied by the number of standards, does not exceed the number of wells on the plate.
- Press **CLEAR** to clear the value on the display.
- Press **ENTER** to continue.

Average Standards

The **AVERAGE STANDARDS** option allows you to select whether or not to average the replicates of each standard group. This average is used to calculate the standard curve instead of using the individual replicates of each standard.

❖ If the number of standard replicates is 1, this option is not available.

```
A V E R A G E   S T A N D A R D S ?   Y E S
  Y E S         N O
```

- Select **YES** to average the replicates for each standard group, and then use the group averages when calculating the standard curve.
- Select **NO** to use the individual standard replicates when calculating the standard curve.
- Press **ENTER** to continue.

Standard Concentrations

The Standard Concentration field allows you to enter the predicted or expected concentration value for each standard group. If **manual mapping** was selected, the replicate locations must also be defined.

```
C O N C N   O F                L O C A T I O N

  S T D 1 : 0                  R E P #   1 : A 0 1
```

- Use the **numeric** and **alpha** keys and the **decimal point** key to enter standard concentration values. The range is 0.00001 to 999999. The entry cannot exceed six characters including the decimal point.

- If automatic mapping is selected, each replicate's location is available for viewing only. Pressing **ENTER** advances to the concentration value entry for the next standard.

- If manual mapping is selected, the location must be defined. Pressing **ENTER** from the standard concentration entry moves the cursor to the **LOCATION** field. Pressing **ENTER** from the **LOCATION** field advances to the concentration value entry for the next standard.

Valid Well Locations

When defining the replicate locations, only the **alpha** keys are active for the first character and **numeric** for the second and third characters. Valid characters and numeric entries are based on the selected plate geometry. The following table lists acceptable entries for well locations based on plate geometry:

Plate Type	Range
6-Well	A01-B03
12-Well	A01-C04
24-Well	A01-D06
48-Well	A01-F08
96-Well	A01-H12
96H	A01-H12
96M	A01-H12

Reuse of Standard Curves

The ELx800 has the ability to reuse a standard curve that has already been established.

Limitations of the Reuse of Standard Curves

- Standard curves can only be reused in assay positions 31 through 55. Each of these positions can only store one standard curve.
- Standard curves cannot be reused on panels (see page 85 for Panel Definition).
- Standard curves will be stored in memory with the Assay Name, Standard Concentrations, Replicate Counts, and Optical Density values for each standard replicate.
- Stored standard curves can only be reused for the assay that the curve was originally generated on (e.g., the curve for Assay 53 cannot be applied to samples on a plate to be run in Assay 51).
- To reuse a standard curve, you must first program an assay (in positions 31 through 55) and then run the assay. During the defining process, you will be prompted to enter the number of standards, the number of standard replicates, and the standard concentrations. The following screen will appear after these prompts:

```
R E U S E   S T A N D A R D   C U R V E ? Y E S

  Y E S       N O
```

After the assay has been run, the results have been calculated, and the reports have been generated, the reader will prompt if this standard curve should be stored in memory. The following display will appear:

```
S A V E   S T A N D A R D   C U R V E ?   Y E S

  Y E S       N O
```

Select **YES** to store the curve for use at a later time. The next time a plate is to be read using this assay, the instrument will prompt if there are standards on the plate. Select **NO** to discard the curve.

```
S T A N D A R D S   O N   P L A T E ?     N O

Y E S   N O
```

If **YES** is chosen, a new standard curve will be generated. The plate map is not changed. (If "Prompt for Sample ID" is enabled in the UTIL section, you will be prompted to enter the number of samples. See **Other Utility Options** in **Chapter 3** for more information on the UTIL options.)

If **NO** is chosen, the stored standard curve will be used. If **Auto mapping** had been used to originally map the standards, blanks, controls and samples defined for this assay, the map will be automatically regenerated without the standards, beginning in well xxx (where xxx was chosen as the starting well in the map, usually well A01). If **manual mapping** was used to map the plate, the map is ***not*** regenerated – the reader will ***not*** produce results for the well positions that originally were standards. Auto mapping is recommended, if the standard's curves will be routinely reused.

Number of Controls

The **NUMBER OF CONTROLS** screen allows you to enter the number of control ***groups*** that will be used in the assay. Any previously defined value will appear on the display.

```
E N T E R   N U M B E R   O F

C O N T R O L S :                    0 2
```

- Use the **numeric** keys to enter the **NUMBER OF CONTROLS** ***groups*** in the assay. For example, if the assay requires one or more positive control wells and one or more negative control wells, enter 02.
- The valid entry range depends on the number of locations on the plate that are undefined. The maximum number of control groups is 8.

Control Type

This option allows you to enter the type of control used in the assay. Any previously defined Control Type will be displayed on the screen.

```
C O N T R O L #   1 :                P C

   P C         N C     H P C         * M O R E
```

```
C O N T R O L #   1 :                P C

  L P C       C T L 1       C T L 2   * M O R E
```

```
C O N T R O L #   1 :                P C

  C T L 3     C T L 4                 * M O R E
```

- Choose one control identifier for each type of control in your assay. The available options are: **P**ositive **C**ontrol**, N**egative **C**ontrol, **H**igh **P**ositive **C**ontrol, **L**ow **P**ositive **C**ontrol, **CTL1**, **CTL2**, **CTL3**, **CTL4**.
- After choosing an identifier for **CONTROL# 1**, press **ENTER** to choose the identifier for the next control.

Number of Control Replicates

The **NUMBER OF CONTROL REPLICATES** entry screen is displayed if the number of control groups is greater than 0.

```
E N T E R   N U M B E R   O F

R E P L I C A T E S   O F   P C :          0 2
```

- The well ID associated with **CONTROL# 1** appears first. Press **ENTER** to advance to the next control.
- Use the **numeric** keys to enter a value for Number of [Control] Replicates.
- The valid entry range is from 1 to 12 replicates. The software automatically performs a check to ensure the number of replicates, multiplied by the number of controls, does not exceed the number of undefined wells remaining on the plate.

Location of Controls

Use this option to enter the location of controls in the assay.

```
C O N T R O L # 1              L O C A T I O N

T Y P E : P C                   R E P # 1 : A 0 2
```

> ❖ The displayed location field can only be edited if **manual mapping** was selected (see page 54). This screen is not accessible in **auto map**.

- Use the **numeric** and **alpha** keys to enter the well location for Rep #1 of Sample Group #1. Press **ENTER** to advance to the next replicate or sample group.

Number of Samples

The number of sample groups on the plate can be defined here, and/or it can be defined at run-time if **UTIL ➔ READ ➔ PROMPT FOR SAMPLE COUNT?** is set to **YES**. See Selecting Read Options on page 101 for more information.

```
E N T E R   N U M B E R   O F

S A M P L E S :                          2 4
```

- Use the **numeric** keys to enter the number of sample ***groups*** on the plate.
- The range is 0 to the number of undefined well locations remaining on the plate. For example, if there are no controls, blanks, or standards defined on a 96-well plate, the maximum number of samples is 96, and the minimum number of samples is 1.

Number of Sample Replicates

After the number of sample groups is entered, the **NUMBER OF SAMPLE REPLICATES** entry screen is presented.

```
E N T E R   N U M B E R   O F
S A M P L E   R E P L I C A T E S :        0 2
```

- Use the **numeric** keys to enter the number of sample replicates.
- The valid range is from 1 to 12 replicates. The software automatically performs a check to ensure that the number of replicates multiplied by the number of samples does not exceed the number of undefined wells remaining on the plate.

Sample Location

If **MANUAL MAP GENERATION** is selected and samples are defined, the locations for each sample replicate must be specified.

```
S A M P L E   # 1          L O C A T I O N
                           R E P #   1 : C 0 2
```

- Use the **numeric** and **alpha** keys to enter the well location for Rep #1 of Sample Group #1. Press **ENTER** to advance to the next replicate or sample group.

Defining FORMULA

The ELx800 supports three types of formulas (Cutoff, Transformation, and Validation), as well as the ability to program variables for use within formulas. Up to three types of Validation formulas may be defined (Blank, Control, and Assay Validation).

❖ Formula definition is not available in the EL800 version.

To define formulas:

1. Start at the Main Menu and select **DEFINE**.
2. Select the assay then press **ENTER** to display the **DEFINE** options screen.

```
D E F I N E :

M E T H O D   M A P   F O R M U L A   C U R V E
```

3. Select **FORMULA**. The **SELECT FORMULA TYPE** screen will appear (described on the next page).

Calculation Structure

During data reduction, formulas are processed in the order shown below. The number of permitted formulas of each type is shown as well.

- Blank Validation 0-1
- Control Validation 0-4
- Assay Validation 0-4
- Transformations 0-1
- Cutoff Formulas 0-1
- Curve-Fit Analysis (if a curve-fit method is defined)

To capture and manipulate the raw data using 384-well microplates with the ELx800NB, you must use KCjunior™ or KC4™. KCjunior and KC4 are PC-based software programs you can use to set up your assay, communicate with the ELx800 to run the assay, and then manipulate the raw data that is automatically retrieved from the reader. See Refer to **Appendix B** for additional information.

Formula Type

The ELx800 supports three types of formulas, as well as the ability to define variables for use within Transformation formulas.

❖ **Note: GENERAL** formulas are not used.

```
S E L E C T   F O R M U L A   T Y P E :
C U T O F F   T R A N S   V A L       * M O R E
```

```
S E L E C T   F O R M U L A   T Y P E :
G E N E R A L   T R A N S - V A R     * M O R E
```

- **CUTOFF** formulas are used to classify results. During data reduction, results are evaluated against the cutoff formulas, and each well is assigned a user-specified label (**POS**, **NEG**, or **EQUIV**).
- **TRANS**formation formulas are applied to the raw data in preparation for further data reduction and/or curve fit calculation.
- **VAL**idation formulas can be used to determine whether or not blanks and/or controls are valid. In addition, Assay Validation formulas can be used to determine whether or not the entire assay should be considered valid.
- The **TRANS-VAR** option allows you to define a variable to be used in transformation formulas.

Formula Entry

After the formula type is selected, the **FORMULA ENTRY** screen appears. Each formula can contain a maximum of 24 characters. Spaces are not necessary.

❖ **Note:** In formulas, "OD" is used to represent the optical density value.

```
F O R M U L A # 1 :

  M A T H   O T H E R     M A P     F U N C T N
```

```
P C ; X > 1 . 2 0 0

  M A T H   O T H E R     M A P     F U N C T N
```

- After a moment, the **FORMULA #1:** prompt disappears, and the formula can be entered. Use the options found under **MATH**, **OTHER**, **MAP**, and **FUNCTN** to "build" the formula.
  - To cycle through the available **MATH, OTHER, MAP,** or **FUNCTION** options, continue to press the appropriate **SOFT KEY.** For example, press the **MATH SOFT KEY** several times to see +, -, *, /, %, =, etc. When the desired option appears, press the **right arrow** key to select it and advance to the next editable field.
  - Press the **left arrow** key to move the cursor to the left.
  - Press **CLEAR** to delete the item above the cursor.
  - When a formula is complete, press **ENTER** to continue.
- Select **MATH** to insert a mathematical symbol such as +, %, or <=.
- Select **OTHER** to insert an opening " (" or closing ") " parenthesis, or logical operators **AND** or **OR**.
- Select **MAP** to insert a well ID such as **BLK;x or NC;1**.
- Select **FUNCTN** to insert a mathematical function such as **LOG** or **SQRT**.

The reader software checks the formulas for errors during data reduction. A syntax error in a formula will result in a "Token Error" on results reports.

MATH

The following mathematical symbols can be used in formulas:

+	Addition
-	Subtraction
*	Multiplication
/	Division
%	Percent

==	Equal to
>	Greater than
>=	Greater than or equal to
<	Less than
<=	Less than or equal to

OTHER

The following additional symbols can be used in formulas:

(	Left parenthesis
)	Right parenthesis
AND	Logical AND
OR	Logical OR

MAP

The available **`MAP`** options depend on the formula type and the current plate map. **`MAP`** options resemble **`BLK;x`** (mean of the blank wells), **`NC;1`** (the first NC well), or **`OD`** (every well).

FUNCTION

The following functions can be used in formulas:

LOG10	Log Base 10
ALOG10	Anti Log Base 10
AB	Absolute Value
PWR	Power

ALOG	Anti Log
LOG	Log
SQRT	Square Root

EXAMPLES

LOG10- Log Base 10-
Log10 2 = 0.301029995

ALOG10- Anti Log Base 10-
ALOG10 (0.30102995) = 2

AB- Absolute Value- AB (-1) = 1

PWR- Power- (10 PWR 2) = 100

ALOG- Anti Log- ALOG (0.69314718) = 2

LOG- Log- LOG 2 = 0.69314718

SQRT- Square Root- SQRT 2 = 1.4142

Validation Formulas

Validation formulas can be used to determine whether or not blanks and/or controls are valid. In addition, Assay Validation formulas can be used to determine whether or not the entire assay should be considered valid.

See **Formula Type** on page 70 for instructions on selecting an assay and accessing the **SELECT VALIDATION TYPE** screen.

```
SELECT VALIDATION TYPE:

CONTROL          ASSAY     BLANK
```

Control and Blank Validation Formulas

Blank Validation is used to ensure that the OD values for the blank replicates, or for the blank mean, meet certain criteria. Control Validation serves the same purpose as Blank Validation, but it applies to the control replicates or control mean. If the criteria are not met, results are considered suspect, and the message **"RESULTS INVALID! BLANK (OR CONTROL) VALIDATION FAILED"** appears on results reports.

- One blank validation formula can be defined.
- Up to four control validation formulas can be defined.
- Define the plate map (via **DEFINE** → **MAP**) before creating blank or control validation formulas.
- Blank/Control validation can be performed on individual replicates (**BLK, PC**), or on the group mean (**BLK;x, NC;x**).

Examples

If an assay protocol states that

- Each blank well on a plate should have an OD of less than 0.050, the formula is: **BLK < 0.050**
- Each Positive Control replicate must have an OD higher than 1.000, but less than 2.500, this can be accomplished with one formula: **PC>1.000ANDPC<2.500**

  Or with two separate formulas:
  PC > 1.000 *and* **PC < 2.500**
- Negative Control mean must have an OD of less than 0.100, the formula is: **NC;x < 0.100**

Number of Required Controls/Blanks

If a control or blank validation formula is defined, enter the number of valid controls or blanks that must meet the criteria established by that formula.

```
PC:  NUMBER OF VALID
REPLICATES REQUIRED:    02
```

- Use the **numeric** keys to enter the **NUMBER OF REQUIRED CONTROLS**. The range is 1 through the number of defined replicates of a control or blank.
- Press the **CLEAR** key to clear the displayed value.
- Press **ENTER** to save the displayed value and advance to the next screen, or use the **Previous Screen** key to move backward through the menu structure.

Assay Validation Formulas

Assay Validation formula(s) establish a set of criteria used to determine whether or not an assay can be considered valid. If the criteria are not met, results are considered suspect, and the message **"RESULTS INVALID! Assay validation failed"** appears on results reports.

- Up to 4 assay validation formulas can be defined.
- Define the plate map (via **DEFINE** → **MAP**) before creating assay validation formulas.

Examples

If an assay protocol states that for the assay to be valid:

- The mean of the negative controls must be less than 0.100. The formula is: **NC;x<0.100**
- The mean of the positive controls must be greater than the mean of the negative controls. The formula is: **PC;x>NC;x**

Transformation Formulas

Transformation formulas can be used to transform raw or blanked absorbance data in preparation for further data reduction, including curve-fit analysis.

See **Formula Type** on page 70 for instructions on selecting an assay and accessing the Transformation Formula definition screen.

- If a blanking method is selected in the assay, transformation formulas are applied to the blanked absorbance values; otherwise, they are applied to the raw data. Turn to page 69 to review the results calculation structure.
- One transformation formula may be defined per assay.
- A transformation formula can be simple (ex. `(OD/2)*100` to multiply all wells on the plate by 100), or more complex with the inclusion of a predefined Transformation Variable (see *TVAR*, below).

Simple Transformation Formulas

"Simple" transformation formulas are typically applied to all wells on the plate. For example:

- To divide the OD in each well on the plate by 2 and then multiply by 100, the formula is: `(OD/2)*100`

Transformation Variable (TVAR)

For more complex transformations, a Transformation Variable (TVAR) can be defined for use within a transformation formula. This variable defines the scope of the transformation: whether to apply the transformation to all of the wells on the plate (OD), or just to the sample wells (SMP).

```
S C O P E   V A R I A B L E :              O D

  S M P       O D
```

- If **SMP** is chosen:
  - The transformation formula will be applied to the ***sample*** wells only.
  - SMP and any other well identifiers (**BLK, PC, NC, STD,** etc.) defined will become available as MAP options when building the transformation formula.

    Example:

    The assay plate map contains 2 NC wells and 2 PC wells. The remainder of the map is filled with samples.

    The assay data reduction requires that the mean of the NC be subtracted from all the samples on the plate.

    The transformation formula is: **SMP-NC;x**

- If **OD** is chosen:
  - The formula definition screen will appear so that you can define a formula for use *within* the transformation formula.
  - Use the formula keys (Math, Other, Map and Function) to define the Transformation Variable (TVAR). Once the variable has been defined, it can be used in a transformation formula. The TVAR will be available as a MAP option when building the transformation formula.

    Example:

    The assay plate map has 2 blanks, 1 control well in duplicate (**CTL1**), 1 negative control well in triplicate **(NC)**, and 5 standards in duplicate **(STD1-STD5)**.

    The assay data reduction states:

  - Subtract the mean of **CTL1** from the mean of the **NC**. Subtract the difference from all ODs on the plate.
  - Divide the result of the above by the mean of the **NC** less the mean of **CTL1**, and then multiply by 100.

On paper, the formula reads:

(OD-(NC;x-CTL1;x))/(NC;x-CTL1;x)*100

On the reader, the formula **(NC;x-CTL1;x)** will be programmed as the TVAR, since the transformation will apply to all standards, controls, and samples on the plate.

- At the **SCOPE VARIABLE** selection screen, choose **OD** and press **ENTER**.
- Enter the formula **(NC;x-CTL1;x)** by using the **MATH, OTHER, MAP** and **FUNCTION** keys. Press **ENTER**.
- The formula definition screen is displayed. Choose **TRANS**.
- Enter the formula **(OD-(TVAR)/(TVAR)*100)** using the **MATH, OTHER, MAP,** and **FUNCTION** keys. ("TVAR" is available as MAP option.)

Example:

In the case of competitive reactions, converting absorbance data to percent B/B_0 can be: **(OD/STD1)*100**. This divides all the wells by **STD1**, presumably the 0 standard, and multiplies the results by 100. To do this:

- At the **SCOPE VARIABLE** selection screen, choose **OD** and press **ENTER**.
- Enter **STD1** as the TVAR formula. Press **ENTER**.
- The formula selection screen is displayed. Choose **TRANS**.
- Enter the formula **(OD/TVAR)*100** using the **MATH, OTHER, MAP,** and **FUNCTION** keys. "TVAR" is available as a MAP option.

Cutoff Formulas

A cutoff formula calculates a **cutoff value** that is used for classifying samples. See **Formula Type** on page 70 for instructions on selecting an assay and accessing the Cutoff formula definition screen.

During data reduction, results are evaluated against the cutoff value (with an optional greyzone), and each well is assigned a call **`POS`** (positive), **`NEG`** (negative), or **`EQUIV`** (equivocal).

- One cutoff formula may be defined per assay.
- If Transformation Formulas are defined, cutoffs are based on the transformed results. Refer to "Defining Formula" on page 69 for the order in which formulas are processed.
- A cutoff formula can consist of a simple numeric value (**`1.500`**); a well identifier (**`PC`** to indicate the criterion for each of the PC replicates, or **`PC;x`** to indicate the average of the Positive Control replicates); or a formula combining the two (**`NC;x+0.050`**).
- A "greyzone" around the cutoff value can be defined, to indicate equivocal or indeterminate results.
- Do not use the **`<`** or **`>`** mathematical symbols in a cutoff formula.

❖ **Tip:** Choose to print a Column Report to see the greyzone and cutoff values as well as the equations used to assign calls to samples.

Greyzone Entry

The **greyzone** is a definable area around the cutoff value. Samples that fall within an area defined by the greyzone (ex. ± 5.0% of the cutoff value) could be considered equivocal (EQUIV).

```
E N T E R   G R E Y Z O N E :          0 5 %
```

- Use the **numeric** keys to enter the greyzone percentage.
- The valid entry range is from 00 to 99%. An entry of 00% indicates no greyzone, although a sample equal to the cutoff value will still receive the EQUIV call.
- See **Positive / Negative Calls** on the next page for information on how calls are assigned.

Positive / Negative Calls for Cutoff

After the greyzone is defined, calls for the sample wells (**POS**itive, **NEG**ative, **EQUIV**ocal) must be defined.

```
SAMPLE>CUTOFF+05%:        POS

  POS        NEG
```

- Select **POS** or **NEG** to select the call that will be assigned to samples greater than the cutoff value plus the greyzone.
- If, for example, **POS** is selected as shown in the above screen, calls will be assigned according to the following equations (**SMP** represents the sample wells):

EQUIV: **SMP <= (CUTOFF+(CUTOFF*GREYZONE))** and

SMP >= (CUTOFF-(CUTOFF*GREYZONE))

POS: **SMP > (CUTOFF+(CUTOFF*GREYZONE))**

NEG: **SMP < (CUTOFF-(CUTOFF*GREYZONE))**

Examples

1. The cutoff between negative and positive calls should be calculated as the average of the negative controls plus the OD value of 0.500. Samples greater than the cutoff should be labeled as positive. No greyzone is required.
   - For this example, **NC;x** (the mean of the NC wells) equals 1.000 OD
   - The cutoff formula is **NC;x+0.5**
   - The greyzone is **00%**
   - **POS** is selected for **SAMP>CUTOFF+00%**
   - Calls are assigned to sample wells as follows:
     - **EQUIV** if the sample equals 1.500
     - **POS** if the sample is greater than 1.500
     - **NEG** if the sample is less than 1.500

2. For a quantitative assay, samples with OD values greater than the **STD2** mean plus a 10% greyzone should be labeled as positive; samples with OD values less than the **STD2** mean minus the 10% greyzone should be labeled as negative. All other samples should be considered equivocal.

    - For this example, **STD2;x** (the mean of the STD2 wells) equals 2.000 OD
    - The cutoff formula is simply **STD2;x**
    - The greyzone is **10%**
    - POS is selected for **SAMP>CUTOFF+10%**
    - Calls are assigned to sample wells as follows:
        - **EQUIV** if the sample is greater than or equal to 1.800 *and* less than or equal to 2.200
        - **POS** if the sample is greater than 2.200
        - **NEG** if the sample is less than 1.800

Defining CURVE

> ❖ **Note:** This feature is not available in the EL800 version (see Appendix D to compare the EL800 with the ELx800).

To define curve-fitting parameters for an assay:

1. Start at the Main Menu and select **DEFINE**.
2. Select the assay and then press **ENTER**. The **DEFINE** option screen will appear:

```
D E F I N E :

M E T H O D   M A P   F O R M U L A   C U R V E
```

3. Select **CURVE**. The definable curve-fitting parameters include:
   - Curve-Fit Type
   - Editing of Outliers
   - Axis Identification
   - Extrapolation of Unknowns

> ❖ **Note:** These screens are displayed on the ELx800 in the order in which they appear in the assay. If a closed variable (i.e., an element of the assay definition that you cannot access or modify) is being used in the assay, the entry screen is omitted.

Curve-Fit Type

The ELx800 supports seven different curve-fitting methods: linear, quadratic, cubic, 4-P, 2-P, cubic-spline, and point to point.

```
C U R V E - F I T   T Y P E :   L I N E A R

  N O N E   L I N E A R   Q U A D     * M O R E
```

```
C U R V E - F I T   T Y P E :   C - S P L I N E

  C U B I C     4 - P       2 - P     * M O R E
```

```
C U R V E - F I T   T Y P E :   C - S P L I N E

    C - S P L I N E       P T - P T   * M O R E
```

- **Linear curve fit:** A simple best-fit straight line is plotted using the values of the standards.

- **Quadratic or "Quad" curve fit:** A curve fit that uses the quadratic equation "$ax^2 + bx + c = y$" to plot the standard's values. Utilizing this curve, any data point for a standard that deviates from the ideal value will not affect the entire curve.

- **Cubic curve fit:** A curve fit that uses the equation "$ax^3 + bx^2 + cx + d = y$" to plot the standard's values. This type of curve fit is affected even less than the quadratic fit when any particular standard has a poor value.

- **2-P (LOGIT/LOG):** A curve fitted to the standard values, which is characterized by a skewed sigmoidal (S-shaped) plot that eventually becomes asymptotic to the upper and lower standard values. The logistic equation is algebraically transformed to a simpler form in which experimentally determined values are used for the responses at concentrations of zero and infinity. **Note:** Uses linear axis type only.

- **Cubic Spline (C-Spline) curve fit:** A piecewise polynomial approximation consisting of joining a set of data points by a series of straight lines, which is then smoothed by using a cubic fit.

- **4-Parameter Logistic or "4-P":** A curve fitted to the standard values, which is characterized by a skewed sigmoidal (S-shaped) plot that becomes asymptotic to the upper and lower standard values. The 4 parameters are: Left asymptote, Right asymptote, Slope and Value at the Inflection point. This fit is most recommended for immunoassay data, and is more exact than Logit/Log. **Note:** Uses linear axis type only.

- **Point to Point or "PT to PT":** A plot that connects each standard point with a line, with no averaging of the values to "smooth" the curve at each standard.

- Press **SOFT KEYS 1, 2, 3,** or **4** to select the curve-fit type that is displayed above the soft key. Select **`*MORE`** to display additional options.

- Press **ENTER** to save the selection and advance to the next screen.

Edit Standard Outliers

This screen allows you to enable or suppress the editing of standard outliers. After the standard curve has been calculated, one or more standards can be excluded from the recalculation of the curve. Any previously defined edit method is displayed.

```
E D I T   S T D   O U T L I E R S : M A N U A L

  N O N E   M A N U A L
```

- Select **NONE** to suppress the **EDIT STANDARD OUTLIERS** capability for this assay.
- Choose **MANUAL** to enable the capability.
  - If **AVERAGE STANDARDS** is set to **NO**, the individual standard replicates are available for editing. If set to **YES**, the standard ***groups*** are available for editing.
  - After the assay is run and reports are generated, press **REPORT** from the Main Menu. Press **RESULT**, select the assay, and then press **ENTER**. The **EDIT STD OUTLIERS? YES/NO** prompt will appear. See **Editing Standard Outliers** on page 94 for further instructions.

X/Y Axis Type

❖ **Note:** LIN/LIN axes are recommended when using the Cubic Spline fit.

After the curve-fit type is selected, select the X/Y Axis Type.

```
X / Y   A X I S   T Y P E :   L I N

L I N     L I N / L O G   L O G   L O G / L I N
```

- Select the method by which the X- and Y-axes will be scaled.
- This option is not available for the 2-P and 4-P curve-fit types. The X/Y scaling for these curves is always LIN/LIN.

Extrapolation of Unknowns

This screen allows you to choose whether to extrapolate the curve to evaluate samples outside of the absorbance range defined by the standards.

```
E X T R A P O L A T E   U N K N O W N S ? Y E S

  Y E S         N O
```

- Select **YES** to enable extrapolation; otherwise, select **NO**.
- On the printed reports, extrapolated concentrations (RSLT values) are surrounded by **< >** (e.g., **<44.425>**).

If extrapolation is chosen for the Point-to-Point curve fit, unknown concentrations will be extrapolated linearly from the nearest segment of the curve. If the plot includes both increasing and decreasing segments, the curve printout will be labeled "Ambiguous." The resulting values, which actually are extrapolated, may not be indicated as such. All calculated results for an "Ambiguous" curve should be considered unreliable.

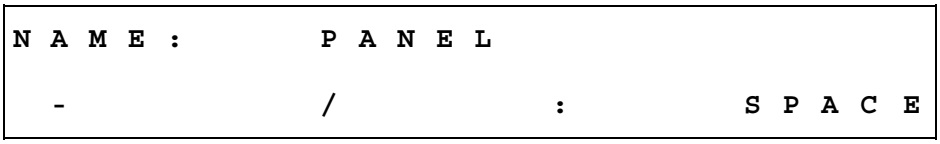
Panel Assays

A Panel assay is a collection of up to 8 assays to be run on one plate.

- The most common reason to use a Panel assay is for confirmatory tests based on a screening test in clinical applications.
- Only one panel can be defined on the reader at any time.
- The assays specified within the Panel must be predefined in any of the assay positions 1-55.
- The assays specified within the Panel must all use the Endpoint read method.
- The assays specified within the Panel must all read at the same wavelength(s).
- Any curve-fit type, formulas, or standard concentrations previously defined for each assay will be used when the assay is selected for a Panel.
- Panel assays cannot reuse standard curves.
- The type and number of controls, blanks, standards, and replicates in the assays chosen for the Panel will be "copied" into the Panel definition. Map or assay parameters must first be changed in the predefined assay before they can change in the Panel.

❖ **Tip:** For use with mapping the Panel, consider printing a Map Report for each assay that will be included in the Panel.

To create a panel assay, start at the Main Menu, select **DEFINE**, and then choose assay number **99**. Enter the panel assay name.



- The default name is **"PANEL"**.
- Use the **alpha** and **numeric** keys to update the assay name, if desired.
- Press **ENTER** to continue. The **NUMBER OF ASSAYS** screen will appear.

```
NUMBER OF ASSAYS:              2
```

- Specify the number of assays to include in the panel (1 to 8).
- Press **ENTER** to continue. The **MAPPING DIRECTION** selection screen will appear.

```
MAPPING DIRECTION: DOWN
DOWN     ACROSS
```

- This option ensures that all assays will be mapped in the same direction.
- Select **DOWN** or **ACROSS**.

The original mapping directions for the predefined assays are overridden by the Panel's mapping directions. If the assay includes replicates, they will follow the Panel mapping direction.

After selecting the mapping direction of the assays, choose which assays to include in the panel.

```
SELECT ASSAY NUMBER:      22
NAME:  HBS-AG1
```

- Press **Options** to cycle through the assay numbers and names, or use the **numeric** keys to enter an assay number. Press **ENTER** to make a selection.
- After an assay is selected, its starting location must be defined.

```
S T A R T   M A P P I N G

A T   W E L L   L O C A T I O N :          A 0 1
```

- Use the **alpha** and **numeric** keys to choose the well location to begin the assay. Wells A01 through H01 are valid for **ACROSS** mapping; A01 through H12 are valid for **DOWN**.

- Repeat this process for each assay within the panel. Remain aware of the total number of controls, standards, and blanks that were originally mapped in each assay while mapping for the panel assay.

- For example, to include Assays 1, 8, and 22 in the Panel assay (**DOWN** mapping is selected for the Panel):

  - Assay 1 has a total of 12 wells defined for controls, blanks, and standards. In the Panel, the mapping for Assay 1 begins in well A01. The user wants to run 6 samples in Assay 1. Assay 1 now fills wells A01 through B03.

  - The mapping for Assay 8 can begin in well B04, or any well other than A01 to B03. The reader will "chirp" if you try to map into a well that is already assigned for use with the Panel.

  - The mapping for Assay 22 may begin at the next available well location after Assay 8 mapping is complete.

  - After all the assays have been entered into the Panel, consider printing the Panel's Map Report to verify the map before reading the plate. Choose **REPORT** (from the Main Menu), **MAP, ASSAY 99**. The reader will print the map of each assay configured in the Panel.

  - The Panel Assay results are sorted by sample (unless a custom assay has been programmed by Bio-Tek).

❖ **Note:** The interpretation of Results reports for each assay in the Panel will print first, and then the Sample results will print.

Reading a Microplate

Use the **READ** option, found at the Main Menu, to select an assay to run, define any required run-time options, and then begin a plate read.

> ❖ **Note:** Before reading a plate, ensure that the reporting options are set correctly under **UTIL** ➔ **OUTPUT**.

To read a plate:

1. Start at the Main Menu and select **READ.**

   The **SELECT ASSAY NUMBER** screen will appear.

2. Select an assay, and then press **ENTER**. See **Selecting an Assay to Run** on the next page for detailed instructions.
3. If required, enter the number of Samples, Plate ID, and/or Sample ID.

> ❖ **Note:** The options to present these screens are configurable by selecting **UTIL** ➔ **READ.** See Selecting Read Options on page 101 for more information.

```
E N T E R   N U M B E R   O F
S A M P L E S :                    2 0
```

```
P L A T E   I D :   1 2 3 4 A
  -           /           :       S P A C E
```

```
E N T E R
S A M P L E   I D :   0 0 0 1
```

4. Place the plate in the carrier, and then press the **READ** key to continue.

Selecting an Assay to Run

To select an assay to run, start at the Main Menu and select **READ**. The **SELECT ASSAY NUMBER** screen will appear:

```
S E L E C T   A S S A Y   N U M B E R :     6 5

N A M E : H B S -A G 1
```

- Use the **numeric** keys to enter the number of any predefined assay stored in the reader's memory, or the **Options** key to advance one assay at a time.
- Press **ENTER** to select the assay and continue.

Run-Time Prompts

After the assay is selected, one or more informational prompts may be presented, depending on preferences selected in **UTIL → READ**, whether or not the assay specifies manual mapping, or if the assay was created or downloaded from Bio-Tek's Extensions™ Define Reader Protocol software.

- Prompts enabled via **UTIL → READ** can include **ENTER NUMBER OF SAMPLES**, **PLATE ID**, and **ENTER SAMPLE ID.**
- If the assay specifies manual mapping, prompts for information will include the locations for the sample wells.
- If running a custom assay, such as one that was created using Extensions™ software, typical prompts might include:
  - The number of samples
  - Standard concentrations
  - Assay ID
  - Fill pattern
  - Blank method
  - First well location
  - Replicate count for each well type
  - Wavelength mode
  - Report preferences, etc.

Enter Number of Samples

If the **ENTER NUMBER OF SAMPLES** prompt is presented, indicate the number of sample ***groups*** on the plate. The number of sample replicates is typically predefined in the assay, but if this is a custom assay, you may also be prompted to enter the replicate count.

```
E N T E R   N U M B E R   O F
S A M P L E S :                    2 0
```

- Use the numeric keys to enter the number of sample groups.
- The valid entry range is from 01 to the maximum number of wells remaining on the plate after any blank, control, or standard wells are mapped.
- If you enter a value greater than the number of empty wells remaining on the plate, the reader will "chirp" and automatically change the value to the maximum permissible number of samples.

Enter Plate ID

If the **PLATE ID** prompt is presented, enter a unique plate identifier to be stored in memory with the assay name and absorbance data.

Use caution when creating multiple Plate IDs. The reader does not warn you that you about to exceed the maximum of 8 plate IDs stored in memory. If a ninth Plate ID is added, it will overwrite the first Plate ID stored in memory.

```
P L A T E   I D :   1 2 3 4 A
  -             /           :          S P A C E
```

- Use up to 10 alphanumeric characters. See page 40 for instructions on using the keypad.
- Press **ENTER** to advance to the **ENTER SAMPLE ID** screen.

Enter Sample ID

If the **ENTER SAMPLE ID** prompt is presented, enter a ***starting*** sample identification number.

```
E N T E R
S A M P L E   I D :   0 0 0 1
```

- The valid entry range is from 0001 to 9999.
- The software will automatically increment each subsequent sample identification number by 1.
- The sample IDs will be assigned according to the plate map defined in the assay.

Prompts for Well Location

If the assay specifies **manual** plate mapping and if **PROMPT FOR SAMPLE COUNT** is set to **YES** under **UTIL → READ,** sample well locations can be defined at run-time.

```
S A M P L E #   1          L O C A T I O N
                            R E P #   1 : G 0 1
```

- The sample well locations originally defined in the assay will be presented. If desired, use the keypad to enter new well locations for each sample replicate.
- Press **ENTER** again to advance to the next replicate.

Beginning the Plate Read

When the following screen appears on the display, the reader is ready to read a plate:

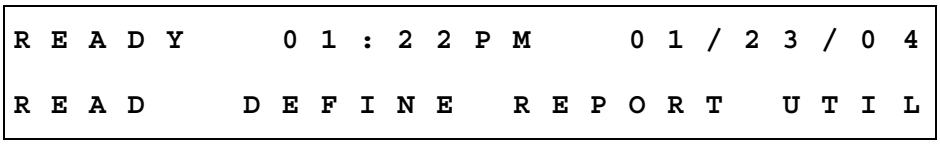
```
P L A C E   P L A T E   I N   C A R R I E R

A N D   P R E S S   < R E A D >   K E Y
```

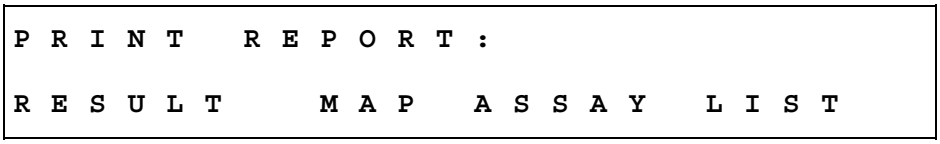
- Before reading the plate, ensure that the printer is connected, turned on, and full of paper.
- Place the plate in the carrier and press the **READ** key to initiate the plate read.
- After the read is complete, data reduction will be performed ("Calculating Results…"), and then the reports will print ("Generating Reports…").
- To halt the read in progress, press the **STOP** key.

Printing Reports

Reports are automatically generated after a plate has been read (see **Specifying Data Output and Reporting Options** on page 99 for information on selecting reports). Results reports also can be regenerated manually by using the **REPORT** option from the Main Menu. In addition, Map, Assay, and Assay List reports can be printed.

❖ **Note:** See **Appendix C** for sample reports.





- Select **RESULT** to print an exact copy of results from the plate reading (the 8
- most recent sets of plate data are stored in memory).
  - The form in which the results are presented is determined by the report settings (Matrix, Column, Curve Fit) specified under **UTIL ➔ OUTPUT**.
- Select **MAP** to print a matrix showing the locations of the Blanks, Standards, Controls, and Samples for a particular assay.
- Select **ASSAY** to print a plate map and a listing of all of the assay's settings, such as wavelengths, numbers of well types, formulas, and curve-fit parameters.
- Select **LIST** to print a list of all assays (name and number) currently programmed in the ELx800.

Results Report

The reader stores the data for the 8 most recent plate reads. Results reports can be generated for these plates if, for example, the data that automatically printed after the read needs to be printed in a different format, or if the standard curve contains outliers that require editing.

```
R E P O R T : H B S - A G

I D :   0 0 1          0 1 / 2 3 / 0 4
```

- The most recently read plate is presented first, showing the assay name, the plate ID (if one was entered), and the date the plate was read.
- Press **Options** to see the next plate in memory.
- Press **ENTER** to select a plate and continue.
- If a standard curve was generated and **EDIT STANDARD OUTLIERS** was set to **manual** in the assay definition, the **EDIT STD OUTLIERS?** prompt is presented; otherwise, the **PRINT RESULTS?** prompt is presented.

Editing Standard Outliers

If a standard curve was generated and if **EDIT STANDARD OUTLIERS** was set to **MANUAL** in the assay definition, the option to edit outliers is presented.

```
E D I T   S T D   O U T L I E R S :

  Y E S     N O
```

- Select **NO** to include all standards in the curve-fit calculations.
- Select **YES** to indicate that one or more standard replicates or groups should be temporarily excluded from curve-fit calculations.
  - If **AVERAGE STANDARDS** was set to **NO** in the assay definition, one or more standard ***replicates*** can be chosen for exclusion.

```
E D I T   S T D 1   R E P 1 ?   Y E S

  Y E S     N O
```

- Select **YES** to exclude the replicate from curve-fit calculations.
- Select **NO** to retain the replicate.
- Press **ENTER** to advance to the next replicate.
  - If **AVERAGE STANDARDS** was set to **YES** in the assay definition, one or more standard ***groups*** can be chosen for exclusion.

```
E D I T   S T D 1 ; X ?   Y E S

  Y E S     N O
```

- Select **YES** to exclude the group from curve-fit calculations.
- Select **NO** to retain the group.
- Press **ENTER** to advance to the next group.

Each curve-fit type requires a minimum number of standards for curve generation: 4 for 2-P, 4-P, cubic, and cubic-spline; 3 for quadratic; and 2 for linear and point-to-point. Exercise caution when editing outliers. If the assay is left with insufficient standards, the curve fit will fail.

Printing Results

After the assay is selected and standard outliers are edited (if necessary), the results report can be printed.

```
P R I N T   R E S U L T S ?

  Y E S     N O
```

- Ensure that the printer is connected, turned on, and filled with paper.
- Press **YES** to print reports, or **NO** to return to the Main Menu.

Map Report

The Map Report contains a matrix in Row x Column format, showing the location of every well identifier defined in the plate map.

```
S E L E C T   A S S A Y   N U M B E R : 0 1

N A M E :   H B S - A G
```

- Press **Options** to cycle through the list of available assays, or enter the number of the desired assay.
- Press **ENTER** to print the report.

Assay Report

The Assay Report lists the assay definition parameters and their current settings.

```
S E L E C T   A S S A Y   N U M B E R : 0 1

N A M E :   H B S - A G
```

- Press **Options** to cycle through the list of available assays, or enter the number of the desired assay.
- Press **ENTER** to print the report.

List Report

The List Report lists the all of the assays (name and number), currently programmed on the reader.

- Select **REPORT** from the Main Menu, and then select **LIST** to print the report.

Using the Utility Options

The **UTILITY** option allows you to set up the date and time, specify your data output and report options, and select your read options.

Setting the Date and Time

To set the current Date and Time, and/or change their formats:

1. From the Main Menu, select **UTIL** → **SETUP.** The **EDIT SETUP INFORMATION** menu will appear:

```
EDIT SETUP INFORMATION:
  DATE    TIME   FILTER  *MORE
```

2. Select **DATE**. The **DATE** entry screen will appear:

```
DATE:     01/23/04            MDY
MMDDYY              DDMMYY
```

   - Enter the new date using the **numeric** keys on the keypad. The cursor is positioned under the first editable field and advances automatically.
   - To change the date format, press the soft key beneath **MMDDYY** or **DDMMYY**. The display automatically updates to reflect the new format.
   - Press **ENTER** to return to the **EDIT SETUP INFORMATION** menu.

3. To change the current time and/or the time format, select **TIME** from the **EDIT SETUP INFORMATION** menu. The **TIME** entry screen will appear:

```
TIME:       12:47PM       12HOUR
12HOUR           24HOUR     AM/PM
```

   - Enter the time using the **numeric** keys on the keypad. The cursor is positioned under the first editable field and advances automatically.
   - To change the time format, select **12HOUR** or **24HOUR**, then **AM** or **PM**. The display automatically updates to reflect the new format.
   - Press **ENTER** to return to the **SELECT UTILITY OPTION** menu.

Viewing/Editing the Filter Table

After installing new filters, ensure that the ELx800's filter table (the reader's software reference for filter locations) matches the physical location of the filters in the filter wheel.

To edit the filter table:

1. From the Main Menu, select **UTIL** → **SETUP.** The **EDIT SETUP INFORMATION** menu will appear:

```
EDIT SETUP INFORMATION:
  DATE     TIME   FILTER   * MORE
```

2. Select **FILTER** to display the wavelength for Filter #1.

```
ENTER
FILTER # 1     WAVELENGTH: 405
```

3. Enter the correct wavelength for each of the five positions, using the **numeric** keys. The range is from 400 to 750 nm (340 to 750 nm for the ELx800NB).

4. Continue to scroll through the five available filter positions, pressing **ENTER** between each.

5. When all five wavelengths have been defined, or skipped, the software will automatically return to the **EDIT SETUP INFORMATION** menu.

Specifying Data Output and Reporting Options

Plate data can be sent to an attached **printer** or external **computer.**

- The onboard software provides several different options for report format and content.
- Data sent to an external computer has no data reduction applied to it, with the exception of dual-wavelength subtraction (if defined in the assay). Any reporting options selected through the onboard software have no effect on serial output.

1. To specify data output and reporting options, start at the Main Menu and select **UTIL** ➔ **OUTPUT.** The **REPORT OUTPUT** screen will appear.

```
R E P O R T   O U T P U T :   B O T H

P R I N T       C O M P U T E R   B O T H
```

2. The current output option is displayed on the top line. Select **PRINT** to send reports directly to a printer, **COMPUTER** to send data out through the serial port, or **BOTH.**

❖ **Note:** These options have no effect on data output if the instrument is being controlled by software (such as KCjunior or KC4) running on a host PC.

3. Press **ENTER** to continue. The **SELECT PRINTER** screen will appear.

```
S E L E C T   P R I N T E R           E P S O N

E P S O N       H P
```

❖ The ELx800 supports printers using either HP's PCL3 language, such as the HP DeskJet series, or Epson's LQ language. For the latest list of compatible printers, consult the Bio-Tek Web site (www.biotek.com), or call Bio-Tek Instruments' Technical Assistance Center (refer to **Chapter 1** for contact information).

4. Select **EPSON** or **HP** as appropriate.

5. Press **ENTER** to continue. The **REPORT TYPE** screen will appear.

```
R E P O R T   T Y P E :          C O L U M N
C O L U M N              M A T R I X     B O T H
```

6. The currently selected report type is displayed in the top line. Select **COLUMN** to print information in a list (columnar) format, **MATRIX** to print in a format that resembles the plate type (ex. 8 x 12 matrix), or **BOTH.**

❖ See **Appendix C** for examples of Reports.

7. Press **ENTER** to continue. The **SAMPLES IN COLUMN REPORT** screen will appear:

```
S A M P L E S   O N   C O L   R P T ?     N O
  Y E S         N O
```

8. Select **YES** to print results for all wells on the plate, including samples.
9. Select **NO** to limit the results information to blanks, controls, and standards.
10. Press **ENTER** to continue. The **PRINT CURVE-FIT** screen will appear.

```
P R I N T   C U R V E - F I T ?           N O
  Y E S         N O
```

11. Select **YES** to print the standard curve (only applies to quantitative assays).
12. Press **ENTER** to return to the **SELECT UTILITY OPTION** screen.

Selecting Read Options

At plate-read time, the software can be configured to present the user with a series of prompts, to enter information such as Plate ID, Sample ID, or Sample Count.

1. To specify various read-time options, start at the Main Menu and select **UTIL ➔ READ.**
2. The **PROMPT FOR PLATE ID** screen will appear. Press **ENTER** to cycle through the prompt screens.

```
P R O M P T   F O R   P L A T E   I D ?   Y E S
  Y E S         N O
```

```
P R O M P T   F O R   S A M P L E   I D ? Y E S
  Y E S         N O
```

```
P R O M P T   S A M P L E   C O U N T ?   Y E S
  Y E S         N O
```

```
R E A D   I N   R A P I D   M O D E ?     Y E S
  Y E S         N O
```

If selected, at read-time:

- **PLATE ID** prompts for microplate identification.
- **SAMPLE ID** prompts for identification for each sample group.
- **SAMPLE COUNT** prompts for the number of samples on the plate.
- **RAPID MODE** prompts for the plate to be read in Rapid Mode or Normal Mode.

❖ **RAPID MODE** reads a 96-well plate in approximately 30 seconds (single-wavelength assay). If **NO** is selected, the plate will be read in **NORMAL MODE** (approximately 50 seconds). Refer to **Chapter 1** for specifications.

- Pressing **ENTER** after each selection advances the display.
- When selections are completed, the display returns to the **SELECT UTILITY OPTION** menu.

Chapter 4

Performance Verification and IQ/OQ/PQ Procedures

This chapter discusses the tasks and procedures necessary for verifying and qualifying instrument performance on an ongoing basis. A convenient Recommended Test Schedule arranges tasks into Installation, Operation, and Performance Qualification categories.

Recommendations for Achieving Optimum Performance

Microplates should be perfectly clean and free of dust or bottom scratches. Use new microplates from sealed packages. Do not allow dust to settle on the surface of the solution; use microplate covers when not reading the plate. Filter solutions to remove particulates that could cause erroneous readings.

Although the ELx800™ supports standard flat, U-bottom, and V-bottom microplates, optimum performance is achieved with optically clear, flat-bottomed wells.

Nonuniformity in the optical density of the well bottoms can cause loss of accuracy, especially with U- and V-bottom polyvinyl microplates. Check for this by reading an empty microplate. Dual-wavelength readings can eliminate this problem, or bring the variation in density readings to within acceptable limits for most measurements.

Inaccuracy in pipetting has a large effect on measurements, especially if smaller volumes of liquid are used. For best results, use at least 100 µl per well in a 96-well plate and 25 µl in a 384-well plate.

Dispensing solution into 384-well plates often traps air bubbles in the wells, which may result in inaccurate readings. A dual-wavelength reading method usually eliminates these inaccuracies; however, for best results, remove the air bubbles by degassing the plate in a vacuum chamber before reading.

The inclination of the meniscus can reduce reading accuracy in some solutions, especially with small volumes. Agitate the microplate before reading to help bring this problem within acceptable limits. Use Tween® 20, if possible (or some other wetting agent) to normalize the meniscus. Some solutions develop menisci over a period of several minutes. This effect varies with the brand of microplate and the solution composition. As the center of the meniscus drops and shortens the light path, the density readings change. The meniscus shape will stabilize over time.

Recommended Test Schedule

The schedule shown in **Table 1** defines the factory-recommended intervals for performance testing for a microplate reader used two to five days a week.

❖ **Note:** The risk factors associated with your tests may require that the Performance and Operational Qualification procedures be performed more or less frequently than shown below.

Table 1

Recommended Test Schedule

	Installation Qualification	Performance Qualification: Monthly	Performance Qualification: Semiannually	Operational Qualification: Initially and Annually
System Self-Test, p. 107	✓	✓		✓
Checksum Test, p. 109	✓			
Absorbance Plate Test, p. 110	✓	✓		✓
Liquid Test 1, p. 119			✓	✓
Liquid Test 2, p.121			✓*	✓*
Liquid Test 3, p. 125 Optional for 340 nm			✓	✓

*Run Liquid Test 2 **ONLY** if you do not have an Absorbance Test Plate. If you run Liquid Test 2, you do **NOT** have to also run Liquid Test 1.

Qualification Procedures

You may use the tests outlined in this section to confirm initial and ongoing performance of the ELx800. Set up the ELx800 according to the instructions in **Chapter 3, Installation**. Confirm that the instrument powers up and communicates with peripherals for the Installation Qualification. After Installation Qualification, conduct the Operational Qualification Tests.

> ❖ **Note:** An Installation-Operational-Performance Qualification (IQ-OQ-PQ) package (PN 7330538) for the ELx800 is available for purchase. Contact your local dealer for more information.

Your ELx800 reader was fully tested at Bio-Tek before shipment and should operate properly upon initial setup. If you suspect that problems may have occurred during shipment, if you reshipped the instrument, or if regulatory requirements dictate that Performance Qualification Testing is necessary, you should perform the following tests. After the initial confirmation of operation, you should perform the Absorbance Plate Test monthly and Liquid Testing semiannually.

- The **System Test** (described on the next page) confirms that the light levels at all installed filter wavelengths and electronic noise meet factory acceptance criteria.
- The **Checksum Test** (page 109) compares the onboard software with internally recorded checksum values to ensure that no corruption has occurred.
- The **Absorbance Plate Test** (page 110) uses Bio-Tek's Absorbance Test Plate to confirm the mechanical alignment, optical accuracy/linearity, and repeatability of the instrument.
- **Liquid Testing** (page 116) uses liquid solutions in a microplate to confirm mechanical alignment, optical accuracy/linearity, and repeatability of the instrument.

Set the ELx800 to **Normal Read Mode** when running all verification procedures (**Chapter 3**).

System Test

IMPORTANT! Do not turn on the instrument until the carrier shipping block has been removed. Refer to **Chapter 2**.

The System Test is performed automatically whenever the instrument is turned on. It can also be performed manually through the ELx800 Main Menu.

The reader will "chirp" repeatedly if the power-on System Test results do not meet the internally coded Failure Mode Effects Analysis (FMEA) criteria established by Bio-Tek. A system test should then be initiated to try to retrieve an error code from the reader.

To run the System Test from the instrument keypad:

1. If running a System Test, attach a printer to the instrument. See **Connecting a Printer to the ELx800** in **Chapter 2** for instructions.
2. From the Main Menu, select **UTIL**. The **SELECT UTILITY OPTION** screen will appear:

```
S E L E C T   U T I L I T Y   O P T I O N :

T E S T S   S E T U P   O U T P U T   R E A D
```

3. From the **SELECT UTILITY OPTION** screen, select **TESTS**. The **SELECT TEST** screen will appear:

```
S E L E C T   T E S T

S Y S T E M   C H K S U M       C A L P L A T E
```

4. Select **SYSTEM** to run the System Test.

   To run the System Test using KCjunior™, open the **Utilities** menu and select **Diagnostics| Reader System Test**.

   To run the System Test using KC4™, open the **System** menu and select **Diagnostics| Run Optics Test**.

   The System Test report lists results in a Pass/Fail format (**Figures 16** and **17**) and can be viewed, saved, and printed. Print the report to document periodic testing or troubleshoot.

❖ If the reader fails the System Test, it will not perform a read until the error condition is cleared. See **Chapter 6, Troubleshooting and Error Codes**, for a list of possible error codes that may be displayed if the System Test indicates a failure.

❖ **Note:** This report may vary slightly if KC4™ or KCjunior™ are used.

```
Operator ID:_____________________________________________________________

Notes:___________________________________________________________________

_________________________________________________________________________

11:00 AM   06/02/03          SYSTEM SELF TEST

Lambda: 200  Gain:  3.28  Resets: 1
Channel:      Ref      1
Air:        20506  35689
Dark:        9911  10002
Delta:      10595  25687

Lambda: 405  Gain:  2.53  Resets: 2
Channel:      Ref      1
Air:        20072  35592
Dark:        9914   9960
Delta:      10158  25632

Lambda: 490  Gain:  1.72  Resets: 2
Channel:      Ref      1
Air:        20280  35642
Dark:        9919   9949
Delta:      10361  25693

Lambda: 550  Gain:  2.51  Resets: 1
Channel:      Ref      1
Air:        20248  35530
Dark:        9914   9985
Delta:      10334  25545

Lambda: 630  Gain:  3.94  Resets: 1
Channel:      Ref      1
Air:        20230  35302
Dark:        9907  10018
Delta:      10323  25284

Lambda: 999  Gain:  5.33  Resets: 1
Channel:      Ref      1
Air:        19977  34186
Dark:        9900  10050
Delta:      10077  24136

Channel:      Ref      1
Noise Max:   9878   9980
Noise Min:   9877   9978
Delta:          1      2
```

Figure 16: Sample output for the System Test (sheet 1 of 2)

```
AUTOCAL ANALYSIS

Upper Left Corner:  x= 9090   y= 10760
Lower Left Corner:  x= 9082   y= 16280
Lower Right Corner: x= 412    y= 16282
Upper Right Corner: x= 416    y= 10762
Delta 1:  9090 - 9082=  +8
Delta 2:   416 -  412=  +4
Delta 3: 10762 -10760=  +2
Delta 4: 16282 -16280=  +2

SYSTEM TEST PASS
```

Figure 17: Sample output for the System Test -- Autocal Analysis (sheet 2 of 2)

Checksum Test

This test also runs automatically when the reader is turned on. The test compares the software to the internally recorded checksum values to ensure that the programming has not been corrupted. It verifies the checksum, and displays the part numbers and versions of software currently loaded onto your reader. If there are any errors during the power-on checksum test, they will be displayed.

1. Press **UTIL** at the Main Menu. At the **SELECT UTILITY OPTION** screen, press **TESTS** and then **CHKSUM** at the **SELECT TEST** screen to run the Checksum Test. The information displayed will ***resemble*** the following:

```
7 3 3 0 2 0 2     V E R S I O N   3 . 0 7

C O D E   C H E C K S U M :          ( 1 7 A 5 )
```

   - The initial checksum test display will show the **onboard (base code) software** part number, version number, and checksum. After a few moments, a second screen will display:

```
7 3 3 0 2 9 1 - F W        V 1 . 0 0 . 0
```

   - The second checksum test display will show the **assay configuration** software part number and version number. After a few moments, the Main Menu will reappear.

Absorbance Plate Test

> ❖ **Note:** Before running the Absorbance Plate test, ensure that the Calibration Plate values are entered. See Entering the Absorbance Plate Specifications in this chapter for more information.

The Absorbance Test Plate or "Test Plate" can be used to conduct several useful tests in a short time period: Mechanical Alignment, Accuracy and Linearity, and Repeatability. The data and results for these tests can be reviewed on the Absorbance Plate Test report. To run an Absorbance Plate Test, you will need a Bio-Tek Absorbance Test Plate (PN 7260522) with its accompanying data sheet.

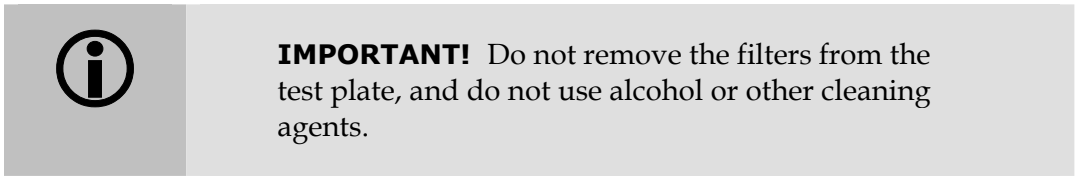
- **Mechanical Alignment:** The Test Plate has precisely machined holes to confirm the mechanical alignment of different microplate readers. The amount of light that shines through these holes indicates whether the reader is properly aligned. A reading of more than 0.015 OD for any of the designated alignment wells indicates that the light is being "clipped" and the ELx800 may be out of alignment. If the reader fails the alignment test, review the following possible problems and solutions:
  - Ensure that the Test Plate is correctly seated in the microplate carrier, with "A1" in the upper left corner. Check the four alignment holes to ensure that they are clear of debris.
  - Check the microplate carrier to ensure that it is clear of debris.

  If these remedies are ineffective, call your Bio-Tek representative or Bio-Tek Technical Support.

- **Accuracy and Linearity:** The Test Plate contains neutral density glass filters that have assigned OD values at several wavelengths. Since there are several filters with differing OD values, the accuracy across a range of ODs can be established. Once it is shown that the device is accurate at these OD values then, by definition, it is linear. Note that there may not be a Pass/Fail indication for filter values that are beyond the specified accuracy range of the instrument.

  If the reader fails the test, review the following possible problems and solutions:

  - Check the filters on the test plate to ensure they are clean. If necessary, clean them with lens paper.



IMPORTANT! Do not remove the filters from the test plate, and do not use alcohol or other cleaning agents.

- Ensure that the filter calibration values entered are the same as those on the Test Plate data sheet.
- Ensure that the Test Plate is within its calibration certification period.

If these remedies are ineffective, or if the Test Plate requires recalibration, call your Bio-Tek representative or Bio-Tek Technical Support.

- **Repeatability:** This test ensures that the instrument meets its repeatability specification by reading each Test Plate neutral density filter twice with the filter in the same location. Note that there may not be a Pass/Fail indication for filter values that are beyond the specified accuracy (and, thus, repeatability) range of the device.

  If the reader fails the repeatability test, review the following possible problems and solutions:

  - Check the filters on the test plate to ensure that there is no debris that may have shifted between readings and caused changes.
  - Check the microplate carrier to ensure that it is clear of debris.
  - If these remedies are ineffective, call your Bio-Tek representative or Bio-Tek Technical Support.

Requirements

To run the Absorbance Plate Test, you need Bio-Tek's Absorbance 7-Filter Test Plate (Part Number 7260522), with its accompanying Data Sheet, shown in **Figure 18**. **Figure 19** shows a sample Test Plate Analysis Report.

This test plate can be used for testing the reproducibility, linearity, and alignment of your Bio-Tek autoreader. The following calibration data has been recorded by a N.I.S.T. traceable spectrophotometer.

WAVELENGTH (nm)

Well	405nm	450nm	490nm	550nm	620nm	630nm	690nm	750nm
C1	0.147	0.140	0.135	0.130	0.136	0.136	0.127	0.134
E2	0.618	0.575				.568	0.485	0.434
G3	1.133	1.052				.040	0.881	0.783
H6	1.701	1.578				.560	1.323	1.179
F5	2.279	2.024	1.976	1.956	1.893	1.865	1.537	1.272
D4	2.945	2.604	2.545	2.513	2.437	2.400	1.972	1.632

SAMPLE

Set # 2453 **Serial # 161259**

Figure 18: Sample Absorbance Test Plate data sheet

❖ **Note:** The Test Plate Analysis report may vary slightly if KC4™ or KCjunior™ are used.

```
Absorbance Test Plate Analysis
Wavelength: 405

Alignment Results
B2= 0.000 PASS   B12= 0.000 PASS   G1= 0.000 PASS   G11= 0.000
PASS

Accuracy Results
                C01    D04    E02    F05    G03    H06
   STANDARD    0.147  2.945  0.618  2.279  1.133  1.701
   DATA        0.139  2.914  0.613  2.265  1.128  1.694
   RESULT       PASS   PASS   PASS   PASS   PASS   PASS

Repeatability Results
                C01    D04    E02    F05    G03    H06
   READ1       0.139  2.914  0.613  2.265  1.128  1.694
   READ2       0.141  2.913  0.616  2.265  1.128  1.694
   RESULT       PASS   PASS   PASS   PASS   PASS   PASS
```

Figure 19: Sample Test Plate Analysis Report

Entering the Absorbance Test Plate Specifications

Using the Data Sheet provided with the Absorbance Test Plate, enter the calibration values.

To enter the values into the reader from the instrument keypad:

1. Start at the Main Menu and select **UTIL** ➔ **SELECT UTILITY OPTION** ➔ **SETUP**. The **EDIT SETUP INFORMATION** menu will appear:

```
EDIT  SETUP  INFORMATION:
 DATE    TIME   FILTER   *MORE
```

2. Press ***MORE** to display the second **EDIT SETUP** menu. Select **CALPLATE** to access the **CALIBRATION FILTER** menu.

3. Using the insert packaged with the Test Plate, select the filter wavelength.

```
EDIT  SETUP  INFORMATION:
RS232    CALPLATE       *MORE
```

```
CALIBRATION  FILTER:  405
 405      450      490      *MORE
```

4. Press **ENTER.** The **WELL LOCATION/CALIBRATION VALUE** screen appears:

```
WAVELENGTH:405  WELL:C01
CALIBRATION  VALUE:  0.158
```

5. Enter the values listed on the Test Plate Data Sheet. After each entry, press **ENTER** to advance to the next consecutive well location. Repeat for the remaining filters.
6. When all values have been entered, press the Main Menu key.

To enter the calibration values into KCjunior™:

1. First define the Absorbance Test Plate to be used for the test. To do this, open the **Utilities** menu, and select **Diagnostics**|**Universal Plate Test**.
2. Click **New Data Sheet**. Click the Help button for guidance.

To enter the calibration values into KC4™:

1. First define the Absorbance Test Plate to be used for the test. To do this, open the **System** menu and select **Diagnostics**|**Define Universal Test Plate| Add Plate**. Click the Help button for guidance.

Running the Absorbance Plate Test

IMPORTANT: Before running the Absorbance Plate Test, ensure that the reader is not running in **Rapid** Mode. To check this, select **UTIL** ➔ **READ** and then cycle through the prompts until **READ IN RAPID MODE?** is displayed. Choose **NO** for an accurate result.

To run the Absorbance Plate Test from the instrument keypad:

1 Start at the Main Menu and select **UTIL** ➔ **TESTS** ➔ **CALPLATE**. The **SELECT TEST** menu will appear:

```
S E L E C T   T E S T :

S Y S T E M   C H K S U M       C A L P L A T E
```

2 Select the appropriate wavelength at the **CALIBRATION FILTER** screen, and press **ENTER** to save the value and continue.

```
C A L I B R A T I O N   F I L T E R :   4 0 5

  4 0 5       4 5 0       4 9 0       * M O R E
```

3 When prompted, insert the Test Plate into the plate carrier, and press the **READ** key to begin the test.

The Calibration Plate Analysis Report (**Figure 19**) will be sent to a printer when the test is run.

To run the Absorbance Plate Test in KC4™:

1. Open the **System** menu and select **Diagnostics|Run Universal Plate Test**.
2. Select the **Universal Plate** and then click **Run Test**.

To run the Absorbance Plate Test in KCjunior™:

1. Open the **Utilities** menu and select **Diagnostics|Universal Plate Test**.
2. Select the **Universal Plate ID** and then click **Run Test**.

Test Failures

If any of the test parameters report as "FAIL," confirm that the standard values on the test plate data sheet match the values on the printout. If not, correct and retest. If the test still fails, contact Bio-Tek's Technical Assistance Center (refer to **Chapter 1** for contact information). Please have a copy of the test and the reader's serial number available when you call.

Liquid Testing

Conducting Liquid Tests confirms the ELx800's ability to perform to specification with liquid samples. Liquid testing differs from testing with the Absorbance Test Plate in that liquid in the wells has a meniscus, whereas the test plate's neutral density glass filters do not. The optics characteristics may differ in these two cases, thus alerting the operator to different types of problems. The liquid tests will help to detect optical defects or contamination that can contribute to errant readings.

- **Liquid Test 1** confirms repeatability and consistency of the reader when a solution is used in the microplate. If these tests pass, then the lens placement and system cleanliness are proven.
- **Liquid Test 2** can be used to test the linearity, repeatability, and alignment of the reader if you do ***not*** have an Absorbance Test Plate. Prepare the series of solutions of varying absorbances as described on page 121.
- **Liquid Test 3** is an optional test, and can only be performed with the "UV" model. It is offered for those sites that must have proof of linearity at wavelengths lower than those attainable with the Absorbance Test Plate. This test is optional since the ELx800™ has good "front end" linearity throughout the specified wavelength range (see page 125).
- Bio-Tek offers a dye solution (PN 7120779, 25 ml; or 7120782, 125 ml) that may be used in the stock solution formulation for Liquid Tests 1 and 2, or, if you prefer, you may use the dye solution described in **Table 3** (page 121). The purpose of the formulation is to create a solution that absorbs light at ~ 2.000 OD full strength when dispensed at 200 µl in a flat-bottom microplate well.
- Alternatively, any solution that gives a stable color will suffice. (This includes substrates incubated with an enzyme preparation and then stopped with an acidic or basic solution.) Some enzyme/substrate combinations that may be used as alternates to the described dye are shown in **Table 2.**

Table 2

Typical Enzyme-Substrate Combinations and Stopping Solutions

Enzyme	Substrate	Stopping Solution
Alkaline Phosphate	o-nitrophenyl phosphate	3N sodium hydroxide
beta-Galactosidase	o-nitrophenyl -beta-D galactopyranoside	1M sodium carbonate
Peroxidase	2,2'-Azino di-ethylbenzothiazoline-sulfonic acid (ABTS)	citrate-phosphate buffer, pH 2.8
Peroxidase	o-phenylenediamine	0.03N sulfuric acid

Stock Solution Formulation

The stock solution for Liquid Tests No. 1 and No. 2 may be formulated from the ingredients listed below (Solution A), or by diluting a dye solution available from Bio-Tek (Solution B).

Solution A

Required Materials:

- Deionized (DI) water
- FD&C Yellow No. 5 dye powder (typically 90% pure)
- Tween® 20 (polyoxyethylene (20) sorbitan monolaurate) **or** Bio-Tek wetting agent, PN 7773002
- Precision balance with readability of 0.001 g
- Weigh boat
- 1-liter volumetric flask

Preparation of Stock Solution:

1. Weigh out 0.092 gram of FD&C No. 5 yellow dye powder into a weigh boat.
2. Rinse the contents into a 1-liter volumetric flask.
3. Add 0.5 ml of Tween 20, or 5 ml of Bio-Tek's wetting agent.
4. Make up to 1 liter with DI water, cap, and shake well.

❖ This should create a solution with an absorbance of about 2.000 when using 200 µl in a flat-bottom microwell. The OD value will be proportional to the volume in the well and the amount of FD&C No. 5 dye used. You can use a larger or smaller well volume, or add more dye or water to adjust the solution. Note that too small a well volume may result in increased pipetting-related errors.

Solution B

Required Materials:

- Bio-Tek QC Check Solution No. 1 (PN 7120779, 25 ml; or 7120782, 125 ml)
- Deionized water
- 5-ml Class A volumetric pipette
- 100-ml volumetric flask

Preparation of Stock Solution:

1 Pipette a 5-ml aliquot of Bio-Tek QC Check Solution No. 1 into a 100-ml volumetric flask.

2 Make up to 100 ml with DI water; cap and shake well.

❖ This should create a solution with an absorbance of about 2.000 when using 200 µl in a flat-bottom microwell. The OD value result will be proportional to the volume in the well and the amount of QC Check Solution No. 1 used. You can use a larger or smaller well volume, or add more Check Solution or water to adjust the stock solution. Note that too small a well volume may result in increased pipetting-related errors.

Liquid Test 1

A 96-well, flat-bottom microplate is required for this test (Corning Costar® #3590 is recommended). Use a new microplate; any fingerprints or scratches may cause variations in readings.

IMPORTANT: Before running the liquid tests, ensure that the reader is not running in **Rapid** Mode. To check this, select **`UTIL`** ➔ **`READ`** and then cycle through the prompts until **`READ IN RAPID MODE?`** is displayed. Choose **`NO`** for an accurate result.

1. Using freshly prepared stock solution A or B (the ***concentrated*** solution), prepare a 1:2 dilution using DI water (one part stock, one part DI water; the resulting solution is a 1:2 dilution).
2. Pipette 200 µl of the ***concentrated*** solution into Column 1 of the plate.
3. Pipette 200 µl of the ***diluted*** solution into Column 2 of the plate.
4. Read the microplate five times at 405 nm using the Normal Read Mode (**Chapter 3**), single wavelength, no blanking.
5. Print the five sets of raw data or export it to an Excel spreadsheet using KCjunior™ or KC4™.

❖ If you are exporting the data to your own Excel spreadsheet, perform the calculations described below and keep the spreadsheet for future tests.

Calculations:

6. Calculate the mean and standard deviation for each well location in columns 1 and 2 for the five plates read. Only one data set needs to be analyzed for each concentration. The well that shows the most variation for each concentration is selected for data reduction.
7. For each mean below 2.000 OD, calculate the allowed deviation using the repeatability specification for a 96-well format of ± 0.5% ± 0.005 OD.

8. The standard deviation for each set of readings should be less than the allowed deviation.

   For example: Absorbance readings of 1.950, 1.948, 1.955, 1.952, and 1.950 will result in a mean of 1.951, and a standard deviation of 0.0026. The mean (1.951) multiplied by 0.5% (1.951 * 0.005) = 0.0098, which, when added to the 0.005 (0.0098 + 0.005) = 0.0148 OD, which is the allowable deviation. Since the standard deviation is less than this value, the reader meets the test criteria.

   Repeatability Specification:

   For comparison in this test, the following repeatability specification is applied, using the Normal Read Mode and a 96-well microplate.

   ± 0.5% ± 0.005 OD from 0.000 to 2.000 OD @ 405 nm

Liquid Test 2

The recommended method of testing the instrument performance is to use the Absorbance Test Plate to confirm alignment, repeatability, and accuracy, which will also confirm linearity.

If a Test Plate is not available, **Liquid Test 2** can be used for these tests.

Required Materials:

- New 96-well, flat-bottom microplates (Corning Costar® #3590 is recommended)
- Ten test tubes, numbered consecutively, stored in a rack
- Calibrated hand pipette (Class A volumetric pipette recommended)
- Stock solution A or B (these are the same solutions as for Liquid Test 1)

Preparation of Dilutions:

1 Set up a rack containing 10 tubes, numbered consecutively.

2 If you have not already done so, prepare concentrated stock Test Solution A or B.

Refer to **Table 3** when executing steps 3 and 4.

Table 3

Test Tube Dilutions

Tube Number	1	2	3	4	5	6	7	8	9	10
Volume of Concentrated Solution (ml)	20	18	16	14	12	10	8	6	4	2
Volume of 0.05% Tween Solution (ml)	0	2	4	6	8	10	12	14	16	18
Absorbance expected if concentrated solution is 2.0 at 200 µl	2.0	1.8	1.6	1.4	1.2	1.0	0.8	0.6	0.4	0.2

3 Create a percentage dilution series, beginning with 100% of the concentrated stock solution in tube 1, 90% of the concentrated solution in tube 2, 80% in tube 3, and so on to 10% in tube 10.

4 Dilute using amounts of the remaining 0.05% solution of deionized water and Tween 20, as shown in **Table 3.**

Plate Preparation:

5 Pipette 200 µl of the concentrated solution from tube 1 into each well of the first column, A1 to H1, of the microplate.

6 Pipette 200 µl from each of the remaining tubes into the wells of the corresponding column of the microplate (tube 2 into wells A2 to H2, etc.).

❖ **Note:** The choice of dilutions and the absorbance of the original solution can be varied. Use **Table 3** as a model for calculating the expected absorbances of a series of dilutions, given a different absorbance of the original solution.

Read Plate

1 Read the microplate prepared above five times using Normal Read Mode dual wavelength at 450 nm with 630 nm as the blank.

❖ **Note:** Do not discard the plate; you will use it for the Alignment Test.

2 Print the raw data or export it to an Excel spreadsheet using KCjunior™ or KC4™.

❖ If you are exporting the data to an Excel spreadsheet, perform the calculations described below and on the following page, and keep the spreadsheet for future tests.

Linearity Test:

3 Calculate the mean absorbance for each well, and average the means for each concentration.

4 Perform a regression analysis on the data to evaluate linearity.

For example, using Microsoft® Excel:

In a spreadsheet, create two columns labeled X and Y. Enter the actual absorbance values in column X. Enter the expected absorbance values in column Y.

Select **`Tools` ➔ `Data Analysis` ➔ `Regression`**. Identify column X as the "Input X Range" and column Y as the "Input Y Range" and then click OK to perform the analysis.

❖ **Note:** If the Data Analysis command is not available on the Tools menu, you may need to install the Analysis ToolPak in Microsoft® Excel. Consult Excel's help system for assistance.

Expected Results:

Since it is somewhat difficult to achieve high pipetting accuracy when conducting linear dilutions, an R-Square value greater than or equal to 0.990 is considered adequate.

Repeatability Test

1 Calculate the mean and standard deviation for the five readings taken above at each concentration. Only one data set needs to be analyzed for each concentration. The well that shows the most variation for each concentration is selected for data reduction.

2 For each mean below 2.000 OD, calculate the allowed deviation using the repeatability specification for a 96-well format of ± 0.5% ± 0.005 OD from 0.000 to 2.000 OD @ 405 nm.

3 The standard deviation for each set of readings should be less than the allowed deviation.

For example:

Absorbance readings of 1.950, 1.948, 1.955, 1.952, and 1.950 will result in a mean of 1.951, and a standard deviation of 0.0026. The mean (1.951) multiplied by 0.5% (1.951 * 0.005) = 0.0098, which, when added to the 0.005 (0.0098 + 0.005) = 0.0148 OD, which is the allowable deviation. Since the standard deviation is less than this value, the reader meets the test criteria.

Repeatability Specification:

For comparison in this test, the following repeatability spec is applied, using Normal mode, 96-well microplate:

± 0.5% ± 0.005 OD from 0.000 to 2.000 OD @405 nm

Alignment Test

1 Using the prepared plate, conduct a turnaround test by reading the plate with the A1 well in the H12 position five times. This test results in values for the four corner wells that can be used to determine alignment.

2 Calculate the means of wells A1 and H1 in the normal plate position (data is from Linearity Test) and in the turnaround position (from Step 1 above). Compare the mean reading for well A1 to its mean reading when in the H12 position. Next, compare the mean values for the H1 well to the same well in the A12 position. The difference in the values for any two corresponding wells should be within the accuracy specification for the instrument.

   For example:

   If the mean of well A1 in the normal position is 1.902, where the specified accuracy is ± 1% ± 0.010 OD, then the expected range for the mean of the same well in the H12 position is 1.873 to 1.931 OD.

   (1.902 * 1% = 0.019 + 0.010 = 0.029, which is added and subtracted from 1.902 for the range.)

3 If the four corner wells are within the accuracy range, the reader is in alignment.

Accuracy Specifications:

For comparison in this test, the following accuracy specification is applied, using Normal Mode and a 96-well microplate:

± 1.0% ± 0.010 OD from 0.000 to 2.000 OD @ 405 nm

Liquid Test 3 (for "UV" Models Only)

Liquid Test 3 is an optional test offered for sites that must have proof of linearity at wavelengths lower than those attainable with the Absorbance Test Plate. This test verifies operation of the ELx800™ at 340 nm and is optional because the reader has good "front-end" linearity throughout its wavelength range.

Required Materials:

- 340 nm filter installed in the reader
- New 96-well, flat-bottom microplates (Corning Costar® #3590 is recommended)
- Calibrated hand pipette(s)
- Beakers and graduated cylinder
- Precision balance with readability to 0.01 g
- Buffer solution A **or** B

Solution A: 10x Concentrate Phosphate Buffered Saline (PBS)

Required Materials:

- Deionized water
- Ingredients shown in **Table 4**
- β-NADH powder (β-Nicotinamide Adenine Dinucleotide, Reduced Form) Sigma® bulk catalog number N 8129, or preweighed 10-mg vials, Sigma® number 340-110

❖ Store the β-NADH powder according to the guidelines on its packaging.

1 Prepare the stock buffer solution using the ingredients in **Table 4**:

Table 4

PBS 10X Concentrate Solution

KH_2PO_4 anhydrous	0.2 grams
NaCl	8.0 grams
Na_2HPO_4 anhydrous	1.15 grams
KCl	0.2 grams
Tween® 20	0.5 ml
Add DI water to bring to	100 ml

2 Mix 5 ml of the concentrated PBS solution with 45 ml of DI water.

3 Add 10 mg of the β-NADH powder and mix thoroughly.

This is the **10x Concentrate PBS Solution**.

Solution B: Sigma PBS

Required Materials:

- Deionized water
- Tween® 20 (polyoxyethylene (20) sorbitan monolaurate)
- Sigma® P 3563 packets
- β-NADH Powder (β-Nicotinamide Adenine Dinucleotide, Reduced Form) Sigma® bulk catalog number N 8129, or preweighed 10-mg vials, Sigma® number 340-110

❖ Store the β-NADH powder according to the guidelines on its packaging.

1. Prepare a PBS solution using the Sigma powder.
2. In a beaker, mix 50 ml of the PBS solution (prepared from the Sigma powder) with 10 mg of the β-NADH powder and mix thoroughly.

This is the **Sigma PBS Solution**.

Procedure

1. Check the absorbance of a sample of buffer solution A or B at 340 nm on the microplate reader. This solution, which will be referred to as the **100% Test Solution**, will have an optical density (absorbance) of approximately 0.700 to 1.000. This value is not critical, but it should be within this absorbance range.

   If low, adjust up by adding β-NADH powder until the solution is at least at the lower end of this range. Do not adjust if slightly high.

2. Carefully prepare a **75% Test Solution** by diluting 15 ml of the 100% Test Solution:
   - If using the Sigma PBS Solution, use 5 ml as the diluent.
   - If using the 10x Concentrate PBS Solution, mix one part of the concentrate with nine parts of DI water. Use 5 ml of this solution as the diluent.

3. Carefully prepare a **50% Test Solution** by diluting 10 ml of the 100% Test Solution:
   - If using the Sigma PBS Solution, use 10 ml as the diluent.
   - If using the 10x Concentrate PBS Solution, mix one part of the concentrate with nine parts of DI water. Use 10 ml of this solution as the diluent.

4. Pipette the three solutions into a new 96-well microplate:
   - 150 µl of the 100% Test Solution into all wells of columns 1 and 2
   - 150 µl of the 75% Test Solution into all wells of columns 3 and 4
   - 150 µl of the 50% Test Solution into all wells of column 5 and 6

5. Read the microplate five times using Normal Mode, single wavelength at 340 nm, no blanking (or blank on air).

6. Print the five sets of raw data or export it to an Excel spreadsheet using KCjunior™ or KC4™.

❖ If you are exporting the data to an Excel spreadsheet, perform the calculations described on the following page, and keep the spreadsheet for future tests.

Repeatability Test

1 For each well, calculate the mean and standard deviation of the five readings.

2 For each mean calculated in step 1, calculate the allowed deviation using the repeatability specification for a 96-well plate in the Normal Read Mode, which is 1.5% + 0.005 OD from 0.000 to 2.000 OD @ 340 nm (mean * 0.015 + 0.005).

3 For each well, compare the standard deviation calculated in step 1 with the allowed deviation calculated in step 2. The standard deviation should be less than the allowed deviation.

For example:

Absorbance readings of 0.802, 0.802, 0.799, 0.798, and 0.801 will result in a mean of 0.8004 and a standard deviation of 0.0018. The mean multiplied by 1.5% (0.8004 * 0.015) equals 0.012, and when added to the 0.005 (0.012 + 0.005) equals **0.017**, which is the allowed deviation for well A1. Since the standard deviation for well A1 is less than 0.017, the well meets the test criteria.

Linearity Test

1 For each of the three dye concentrations, calculate the mean absorbance for the wells containing that solution (mean of wells A1 to H2, A3 to H4, and A5 to H6).

2 Perform a regression analysis on the data to determine if there is adequate linearity.

Example using Microsoft® Excel:

In a spreadsheet, enter the three mean values in ascending order and label the column as the Y values. Enter 0.50, 0.75, and 1.00 and label the column as the X values.

Select **`Tools` ➔ `Data Analysis` ➔ `Regression`**. Identify column Y as the "Input Y Range" and column X as the "Input X Range" and then click **OK** to perform the analysis.

❖ If the Data Analysis command is not available on the Tools menu, you may need to install the Analysis ToolPak in Microsoft® Excel. Consult Excel's help system for assistance.

Expected Results:

Since it is somewhat difficult to achieve high pipetting accuracy when conducting linear dilutions, an R-Square value greater than or equal to 0.990 is considered adequate.

Chapter 5

Maintenance and Decontamination

This chapter contains procedures for maintaining and decontaminating the ELx800.

Maintenance

A daily maintenance schedule is the best way to ensure accurate performance and a long life for your instrument. Frequent cleaning of the microplate carrier and all exposed surfaces of the instrument will help to reduce the amount of particulates or dust that can cause erroneous readings.

Routine Cleaning Procedure

Warning! Internal Voltage. Always turn off and disconnect the reader from the power supply for all cleaning operations.

Do not immerse the instrument, spray it with liquid, or use a "wet" cloth. Do not allow the cleaning solution to run into the interior of the instrument. If this happens, contact the Bio-Tek Service Department.

Do not soak the keypad—this will cause damage. Moisten a clean cloth with deionized or distilled water and wipe the keypad. Dry immediately with a clean, dry cloth.

Do not apply lubricants to the microplate carrier or carrier track. Lubrication on the carrier mechanism or components in the carrier compartment will attract dust and other particles, which may obstruct the carrier path and cause the instrument to produce an error.

Purpose

Exposed surfaces may be cleaned (not decontaminated) with a cloth moistened (not soaked) with water or water and a mild detergent.

Tools and Supplies

- Mild detergent
- Deionized or distilled water
- Clean cotton cloths

Procedure

1. Turn off and disconnect the instrument from the power supply.
2. Moisten a clean cotton cloth with water, or with water and the mild detergent. Do not soak the cloth.
3. Wipe the plate carrier and all exposed surfaces of the instrument.
4. If detergent was used, wipe all surfaces with a cloth moistened with water.
5. Use a clean, dry cloth to dry all wet surfaces.

Replacing the Bulb

The lamp is rated for an average life of 600 hours. The intensity of the lamp will slowly drop over time until the ***run time self check*** detects a low signal level and the instrument displays an error code.

The bulb should be replaced and aligned at this time (use replacement bulb PN 7330513). For UV and NB readers, use replacement bulb PN 7330516. **Figure 20** shows a detailed view of the lamp assembly.

Warning! The lamp will be hot immediately after the reader has been shut down. Allow the lamp to cool down for at least 10 minutes before attempting to replace the bulb.

To replace the bulb:

1. **Important!** Turn off and unplug the instrument.
2. Turn the instrument over on its back and remove the screws holding on the top cover (see **Figure 3** in **Chapter 2**).
3. Turn the instrument over again and lift the top cover off.
4. Use a Phillips-head screwdriver to remove the optics arm cover.
5. Loosen the hex screws that hold the lamp in place. **(Do not remove the screws.)**
6. Slide the bulb out backward and detach it from the connector at the back of the optics arm.
7. Insert the new bulb into the bulb retention springs.
8. Plug in and power up the unit. The lamp should light, although an error message will appear on the instrument's display because the lamp is not aligned. Press any key to return to the Main Menu.

To align the bulb:

1. From the Main Menu, press **UTIL** to access the **SELECT UTILITY OPTION** screen.
2. At the **SELECT UTILITY OPTION** screen, press **SETUP** ➔ **READ** ➔ ***MORE** until **BULB ALIGN** is displayed. The carrier will move to the bulb alignment position.

```
E D I T     S E T U P

B U L B     A L I G N                  * M O R E
```

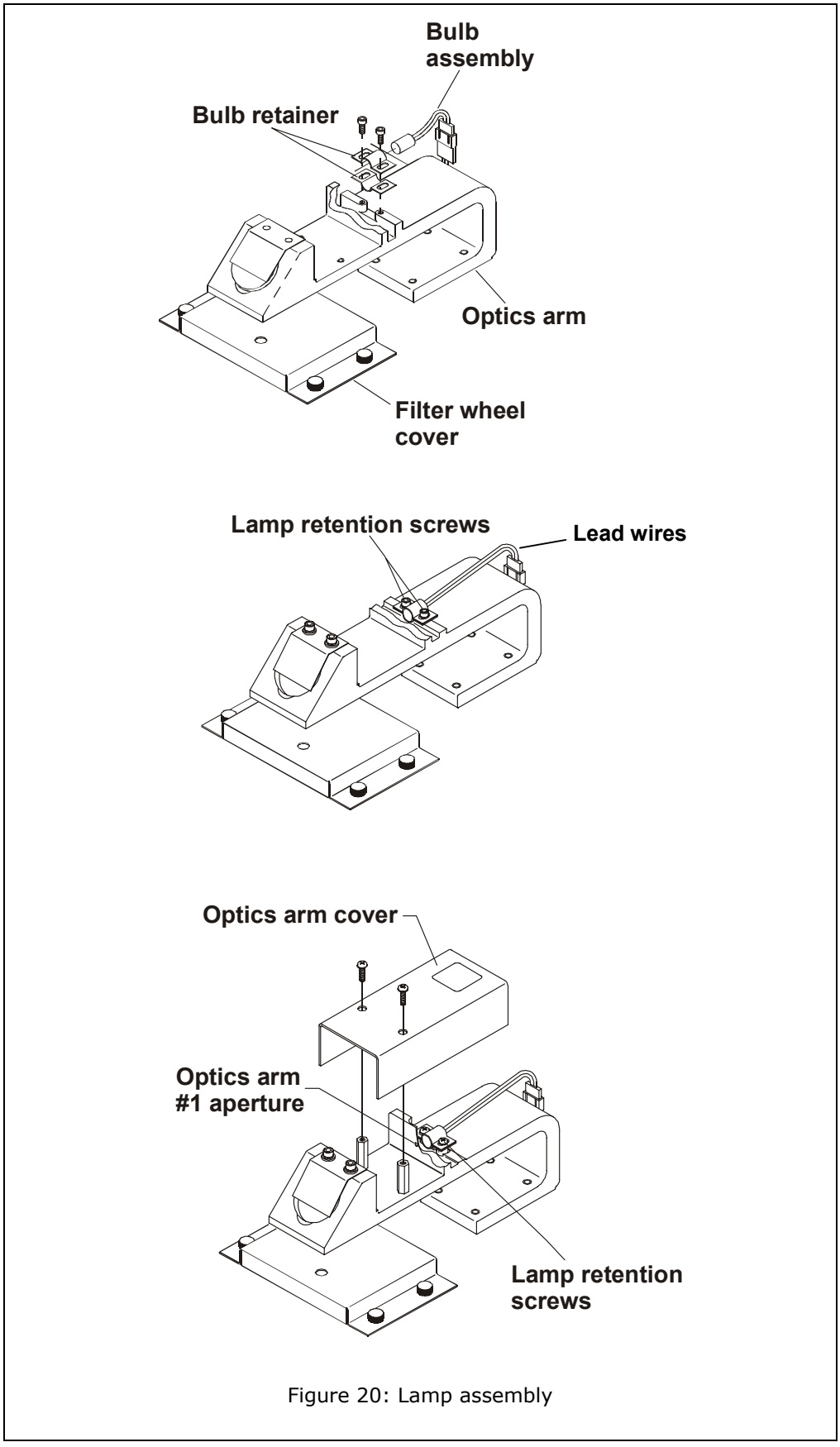


Figure 20: Lamp assembly

To align bulbs on a standard model reader:

Warning! The lamp will be hot immediately after the reader has been shut down. Allow the lamp to cool down for at least 10 minutes before attempting to replace the bulb.

1. Use the lead wires to position the bulb to avoid damaging the glass covering the bulb with fingertips.
2. Push the bulb forward until it stops.
3. Rotate and/or swing the bulb left or right to get a full circular image on the carrier sheet metal.
4. Tighten the lamp retention screws and verify that the lamp has not moved.
5. After completing the alignment, turn the instrument off and on again to return the carrier to the home position.

Warning! The alignment procedure requires you to observe the light path while the bulb is turned on. To prevent possible vision impairment, avoid looking directly at the bulb while it is on.

To align bulbs on UV and NB model readers:

1. Use the lead wires to position the bulb to avoid damaging the glass covering the bulb with fingertips.
2. Keep the tip of the bulb slightly away from the optics arm #1 aperture (1 mm).
3. Rotate and/or swing the bulb left or right to get a good circular image on the carrier's sheet metal.
4. Tighten the lamp retention screws and verify that the lamp has not moved.
5. After completing the alignment, turn the instrument off and on again to return the carrier to the home position.

Decontamination Procedure

Any laboratory instrument that has been used for research or clinical analysis is considered a biohazard and requires decontamination prior to handling.

Decontamination minimizes the risk to all who come into contact with the instrument during shipping, handling, and servicing. Decontamination is required by the U.S. Department of Transportation regulations.

Persons performing the decontamination process must be familiar with the basic setup and operation of the instrument.

Bio-Tek Instruments, Inc. recommends the use of the following decontamination solutions and methods based on our knowledge of the instrument and recommendations of the Centers for Disease Control and Prevention (CDC). Neither Bio-Tek nor the CDC assumes any liability for the adequacy of these solutions and methods. Each laboratory must ensure that decontamination procedures are adequate for the Biohazard(s) they handle.

Wear prophylactic gloves when handling contaminated instruments. Gloved hands should be considered contaminated at all times; keep gloved hands away from eyes, mouth, nose, and ears. Eating and drinking while decontaminating instruments is not advised.

Mucous membranes are considered prime entry routes for infectious agents. Wear eye protection and a surgical mask when there is a possibility of aerosol contamination. Intact skin is generally considered an effective barrier against infectious organisms; however, small abrasions and cuts may not always be visible. Wear protective gloves when performing the decontamination procedure.

Tools and Supplies

- Sodium hypochlorite (NaClO, or bleach)
- 70% isopropyl alcohol (as an alternative to bleach)
- Deionized or distilled water
- Safety glasses
- Surgical mask
- Protective gloves
- Lab coat
- Biohazard trash bags
- 125 ml beakers
- Clean cotton cloths

Procedure

The bleach solution is caustic; wear gloves and eye protection when handling the solution.

Do not immerse the instrument, spray it with liquid, or use a "wet" cloth. Do not allow the cleaning solution to run into the interior of the instrument. If this happens, contact the Bio-Tek Service Department.

Do not soak the keypad—this will cause damage.

Important! Turn off and unplug the instrument for all decontamination and cleaning operations.

1. Turn off and unplug the instrument.
2. Prepare an aqueous solution of 0.5% sodium hypochlorite (NaClO, or bleach). As an alternative, 70% isopropyl alcohol may be used if the effects of bleach are a concern.
   - Be sure to check the percent NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10% NaClO; if this is the case, prepare a 1:20 dilution. Household bleach is typically 5% NaClO; if this is the case, prepare a 1:10 dilution.

3. Moisten a cloth with the bleach solution or alcohol. Do not soak the cloth.
4. Wipe the keypad (do not soak). Wipe again with a clean cloth moistened with deionized or distilled water. Dry immediately with a clean, dry cloth.
5. Wipe the plate carrier and all exposed surfaces of the instrument.
6. Wait 20 minutes. Moisten a cloth with deionized or distilled water and wipe all surfaces of the instrument that have been cleaned with the bleach solution or alcohol.
7. Use a clean, dry cloth to dry all wet surfaces.
8. Discard the used gloves and cloths using a Biohazard trash bag and an approved Biohazard container.

Chapter 6

Troubleshooting and Error Codes

This appendix describes potential error codes that may be displayed on the ELx800, and suggests possible solutions for these problems.

Overview

If an error is displayed, consult the error code list to see if the error is easily correctable. If you cannot resolve the problem, run the instrument **System Test** and note the exact error code and serial number of the reader. Then call Bio-Tek's Technical Assistance Center (refer to **Chapter 1** for contact information).

System Test Description

The System Test feature conducts a series of tests, at each of five set wavelengths, which confirm adequate light levels, low electronic noise, adequate photodiode sensitivity, and overall system cleanliness. The testing is designed to ensure that the ELx800 will give in-specification performance for each set wavelength over the specified OD range.

The reader automatically runs an internal System Test each time it is powered on. The reader will "chirp" repeatedly if the power-on System Test results do not meet the internally coded Failure Mode Effects Analysis (FMEA) criteria established by Bio-Tek. A system test should then be initiated to try to retrieve an error code from the reader.

Glossary of Terms

- **Air Blank:** A full light reading through a filter with no plate in the light path.
- **Dark Current:** A reading taken with the light blocked to measure background light levels in the reading chamber. Also used as a measure of background electronic noise within the measurement circuit.
- **Gain:** An automatic electronic adjustment to the measurement circuit. The gain adjustment compensates for changing light levels or filter variations. For example, if the lamp output decreases slightly, the gain will increase to make up the difference.
- **Axis:** Refers to a motor for the filter wheel or plate carrier.
- **Offset:** A numerical limit, usually a range. For example, if the gain fails an offset test, it may be too high or too low.

Error Codes

An error code is displayed on the instrument as a four-digit identifier. The first character will be 0, 1, 2, or A.

❖ **Note:** If an error code is displayed, run a System Self-Test for diagnostic purposes.

- "0", "1", or "2" indicates a noncritical error, and the instrument will respond to keypad input. See General Errors on the next few pages for more information.

Motor or Optical Sensor	Channel
0 = Carrier X-Axis	0 = Reference
1 = Carrier Y-Axis	1 = Measurement

- "A" indicates a more serious error. In this case, turn off the instrument. Upon restarting the instrument, you should be able to use the keypad. See Fatal Errors on page 148 for more information.

Contact Bio-Tek's Technical Assistance Center for further assistance if any of error codes are displayed. Refer to **Chapter 1** for contact information.

General Errors

General errors indicate nonfatal conditions that require attention.

Code	Description and Probable Causes
0100	**Abort** The read or task has been aborted.
0101	**Abort Error** The read or task has been aborted. The 0101 indicates a software abort. **Probable Causes:** • User aborted read from KC4. • User aborted from another serial interface.
0200	**X-axis (carrier in/out) motor did not find the opto (home) sensor** A motor was unable to move to its "home" position as registered by feedback from an optical sensor. **Probable Causes:** • Defective or broken optical sensor. • Flex cable is disconnected or damaged. • X-axis movement is limited so that the optical sensor cannot be interrupted.

❖ **Note:** In cases where a sensor is not functioning, the motor will drive the axis to its mechanical stop and generate substantial noise.

General Errors (Continued)

0201	**Y-axis (carrier left/right) motor did not find the home sensor** A motor was not able to move to its "home" position as registered by feedback from an optical sensor. **Probable Causes:** • Defective or broken optical sensor. • Sensor not connected. • Y-axis movement is limited so that the optical sensor cannot be interrupted.
0202	**Filter wheel did not find opto (home) sensor** **Probable Cause:** • Failed lamp.
0300-0301	**Light beam not found** During the X-axis movement, the light beam (saturation) transition (max light to no light) was never found during autocalibration. **Probable Causes:** • Loose belt, loose motor pulley, or defective motor drive causing the carrier to ignore movement instructions. • Failed lamp. • Loose filter wheel drive gear or defective motor controller PCB.
0302	**Filter wheel failed positional verify; lamp failed** • A failed lamp generates a 0302 error on power-up.

General Errors (Continued)

0400	**Carrier X-axis movement failed positional verify** Motor X-axis failed to get to the same position when moved a known number of steps from the home position and back. **Probable Causes:** • The belt has slipped because of incorrect tension, a loose motor pulley, or a loose belt clamp. • Defective motor drive.
0401	**Carrier Y-axis movement failed positional verify** Motor X-axis failed to get to the same position when moved a known number of steps from the home position and back. **Probable Causes:** • The belt has slipped because of incorrect tension, a loose motor pulley, or a loose belt clamp. • Defective motor drive.
0402	**Filter wheel failed positional verify; lamp failed** **Probable Causes:** • Optical trigger flag has moved or is loose. • Filter wheel is binding against the motor gear. • Defective motor drive. • Bearings caused motors to fail.
0500	**Filter not installed** **Probable Causes:** • Empty filter location on the filter wheel (all filter locations must have either a filter or a filter blank, Bio-Tek PN 2872086). • Entire filter wheel is not installed.

General Errors (Continued)

0601	**Filter #1 Gain out of range**
0602	**Filter #2 Gain out of range**
0603	**Filter #3 Gain out of range**
0604	**Filter #4 Gain out of range**
0605	**Filter #5 Gain out of range**
	These errors indicate that the gain for the filter in question is out of the range necessary to ensure performance to specifications. **Probable Causes:** • Defective interference filter. • Defective lamp providing reduced signal, causing the error. • Misaligned optics.
0700	**Reader failed noise test** Significant variations in background electronic noise were detected when blocking the light and increasing the gain to maximum. **Probable Causes:** • External signals may be penetrating the measurement chamber. Ensure that the bottom and top shrouds, as well as the filter wheel cover, are correctly installed. • A faulty motherboard (PN 7330400, 7330410, or 7330415) or daughter board (PN 7330404 or 7330414). • Photodiode or power supply may be too close to the instrument. • Failure indicates excessive variation in the dark current (background) noise levels of the measurement circuit.
0800	**Reader failed offset test** During self-test, the background electronic signal detected is outside of acceptable limits at maximum gain when blocking the light. **Probable Causes:** • If noise Max is > 20000. ➢ There may be an ambient light leak. Ensure that the plate carrier door is properly closed. ➢ Faulty motherboard (PN 7330400, 7330410, or 7330415) or daughter board (PN 7330404, or 7330414). ➢ Photodiode or power supply may be too close to the instrument.

General Errors (Continued)

0900	**Read time dark value out of range** • The dark current value taken during the current read is significantly different from the same reading taken during the power-up self-check. **Probable Cause:** • The measurement electronics background noise has changed since the last power-up self-check. ➢ Ambient light may have increased since power-up.
0A01-0A05	**Read time Air Blank out of range** The blank (full signal) reading taken during the current read has changed significantly from the same reading taken during the power-up self-check. **Probable Cause:** • Lamp is unstable. Reboot system and/or install new lamp. Turn unit ***OFF*** when not in use to conserve lamp life.
0B00	**Invalid assay** An assay number that is not programmed was selected.
0C00	**Printer timed out** The time allotted for the instrument to make a valid connection to a printer has expired. **Probable Causes:** • Printer not connected, on line, or powered up. • Printer parallel port may not be correctly selected.

General Errors (Continued)

❖ Errors **0D00** through **1300** indicate that the reader has failed one or more of a number of self-tests. Contact Bio-Tek's Technical Assistance Center for instructions. Refer to **Chapter 1** for contact information.

0D00	**Calibration checksum error** Reader failed calibration checksum test. **Probable Cause:** • The stored checksum value for the calibration data does not match the actual checksum.
0E01-0E05	**Wavelength not found in table** The specified wavelength is not detected in the instrument's filter table. The last number is the filter set number in the assay protocol. **Probable Causes:** • Assay wavelength does not match the filter table. ➢ Run system test. Verify that wavelengths in the filter wheel match settings in the filter table (see **Chapter 2**).
0F00-0F05	**Filter signal out of range** The filter (1-5) has a signal that is out of range. **Probable Causes:** • This indicates a light level problem with one or more filters. • A "UV" filter may have been installed in a non-UV instrument. • A blank filter may be incorrectly installed in a filter position. ➢ Verify that filters in the filter wheel match settings in the filter table (see **Chapter 2**).
1000	**Configuration data missing** Required reader configuration data was missing at the beginning of the self-test. **Possible Causes:** • Necessary configuration data was not downloaded or was downloaded incorrectly.

General Errors (Continued)

1100	**Failed configuration checksum test** The stored checksum value from the configuration data does not match the actual checksum of the current configuration data. **Probable Cause:** • The configuration data has changed and the checksum stored is no longer valid. The error is produced when outdated versions of assay definition software are used to create an assay configuration file. • This file is incompatible with the operation code within the instrument's memory. ➢ Recreate the assay definition using the correct version of assay definition software and re-download the software.
1200	**Autocalibration data missing** No Autocal data exists for the read location. **Probable Cause:** • Autocalibration was not performed following assay definition download.
1300	**Motor not homed successfully** If the motor is not correctly homed, the instrument sends an error and exits the function. **Probable Causes:** • Errors 0200 or 0300 have been ignored. Refer to **Probable Causes** for these errors on pages 142 and 143.

Fatal Errors

Fatal Errors indicate conditions that require immediate attention. If a fatal error is displayed, contact Bio-Tek's Technical Assistance Center for further instructions. Refer to **Chapter 1** for contact information.

Code	Description and Probable Causes
A100	**Task control block not available**
A200	**Read already in progress**
A300	**Device not available**
A304	**Printer device not available**
A400	**Failed code checksum on power-up**
A500	**Code flash readback timed out**
A600	**Quick flash configuration timed out**

Appendix A

Controlling the Reader With KCjunior™ or KC4™

The ELx800 can be controlled either from Bio-Tek's KCjunior™ or KC4™ PC Software. This Appendix provides instructions for programming the computer to control the reader.

Overview

Using KCjunior™ or KC4™ software provides the user even more power and flexibility. For example, complex assays using features not available through the front panel may be defined and run.

This Appendix provides instructions for controlling the reader with Bio-Tek's KCjunior™ or KC4™ software packages.

Controlling the Reader With KCjunior™

The ELx800 can be operated using a computer running Bio-Tek's KCjunior software. Follow the steps below:

1. Power off the computer and the reader. Connect the serial cable (PN 75053) between the two machines.
2. Power up both machines.
3. Install KCjunior on the computer's hard drive.
4. Once installed, start KCjunior.
5. Select **Setup**, then **Reader1.** To select the reader and define the communications parameters, choose the following setup parameters:
   - Reader: ELx800, EL800, or ELx800UV, EL800UV, or ELx800NB, EL800NB
   - Com Port: COM1 or COM2 (the serial port used for the RS-232 cable connection)
   - Baud Rate: 2400, 4800, or 9600 must match the baud rate on the reader)
   - Data Bits: 8
   - Parity: None
   - Stop Bits: 2
   - EOT Character: Keep the default number.
6. Click the **Test Communications** button to attempt to establish communications with the reader, using the currently defined communication parameters. If a **Serial Write Error** dialog box is displayed, an incorrect Com Port may have been selected. Select a different port and then repeat this step.
7. If the test passes, click **OK** to save the settings and close the dialog box. If the test fails, follow the directions provided by KCjunior, then click **Test Communications** again.

To learn more about KCjunior and how to define assays and read plates, refer to the KCjunior User's Manual.

Problems

If KCjunior fails to communicate with the reader, and displays a serial communications error, check the cable plug-in location to make sure it matches the setup choices and is not a Null cable. If this is suspected, add another Null and try again.

Getting Started With KCjunior™

The following instructions *briefly* describe how to read a plate using KCjunior. Refer to KCjunior's Help system and User's Guide early and often to learn how to create protocols, assign well identifiers, read plates, print reports, and more.

To read a plate using KCjunior:

1. Click **Read Plate** from KCjunior's main screen. The **Read Plate** Dialog will appear.
2. If desired, enter a Results ID and a Plate Description, and then click **Read Plate.** The **Protocol Definition** dialog will appear.
3. Select a **Read Method Type** of **Endpoint**, **Kinetic**, or **Multiwavelength.**
4. Define the wavelength(s) at which the plate will be read.
5. Select a **Plate Geometry** from the drop-down list.
6. Define other reading parameters as necessary. Click the Help button for assistance.
7. When complete, click OK to return to the **Read Plate** dialog. If desired, enter a **Plate ID.**
8. Place the plate on the carrier, then click **OK** to start the plate read.
   - The plate will be read and then the raw data results will display in KCjunior. Print the raw data by selecting Plate|Print Results.
   - To analyze or manipulate results, a protocol should be defined. Refer to KCjunior's Help system or User's Guide for instructions.

Controlling the Reader With KC4™

The ELx800 can be operated using a computer running Bio-Tek's KC4 software. Follow the steps below:

1. Power off the computer and the reader. Connect the appropriate serial cable
(PN 75053) between the two machines.
2. Power up both machines.
3. Install KC4 on the computer's hard drive.
4. Once installed, start KC4.
5. Select System, Readers.
6. Scroll through the list of **Available Readers** and select the appropriate ELx800 reader model. Click the **Port** button (and subsequent **Setup** button), to define the following communications parameters:
   - Port: **COM1, 2, 3,** or **4** (the serial port used for the RS-232 cable connection)
   - Baud Rate: **2400, 4800,** or **9600** (must match the baud rate on the reader)
   - Data Bits: **8**
   - Parity:**None**
   - Stop Bits: **2**
7. Click the **Current Reader** button to attempt to establish communications with the reader, using the currently defined communication parameters.
8. If the test passes, click **OK** to save the settings and close the dialog box. If the test fails, KC4 will provide appropriate instructions for resolving any problems. See also the Problems section below.

To learn more about KC4 and how to define assays and read plates, refer to the KC4 User's Manual.

Problems

If KC4 fails to communicate with the reader and displays a serial communications error, check the cable plug-in location to ensure that it matches the setup choices and is not a Null cable. If this is suspected, add another Null and try again.

If an **Incorrect Reader Model Connected** dialog box is displayed, click **OK** to clear the screen and select **System, Readers, Available Readers.** Verify that the reader selected is correct.

Getting Started With KC4™

The following instructions briefly describe how to read a plate using KC4. Refer to KC4's Help system and User's Guide early and often to learn how to create protocols, assign well identifiers, read plates, print reports, and more.

To read a plate using KC4:

1. Select **Data|New Plate.**
2. If prompted to select a protocol, select "Empty Protocol" and click OK. If not prompted, select **Protocol|New,** or use KC4's Protocol Wizard to step through protocol creation.
3. Select **Protocol|Reading.** The **Reading parameters** dialog will appear.
4. Select a **Reading Type** of **Endpoint,** or **Kinetic.**
5. Define the wavelength(s) at which the plate will be read.
6. Select a **Plate Type** from the drop-down list.
7. Define other reading parameters as necessary. Click the **Help** button for assistance.
8. When complete, click **OK.**
9. Select **Data|Read Plate.** The **Plate Reading** dialog will appear.
10. Enter any comments, place the plate on the carrier, then click **Start Reading** to begin the plate read.
    - The plate will be read and then the raw data results will display in KC4.
    - To analyze, manipulate, or print results, **protocol** parameters should be defined. Refer to KC4's Help system or User's Guide for instructions.

Appendix B

Using 384-Well Geometry

The following describes how to manipulate data received from a plate with 384-well geometry. To capture and manipulate the raw data using 384-well geometry, you must use KCjunior™ or KC4™. The Optical Density values will be downloaded, via the RS-232 port, and added to the data logging program.

KCjunior™

KCjunior, Bio-Tek's data reduction package for Windows®, can control the ELx800 in 6-, 12-, 24-, 48-, 96-, and 384-well formats, as well as in 60-, 72- ,and 96-well Terasaki plate formats.

1. Start KCjunior.
2. At KCjunior's main menu, choose **Setup|Reader 1**.

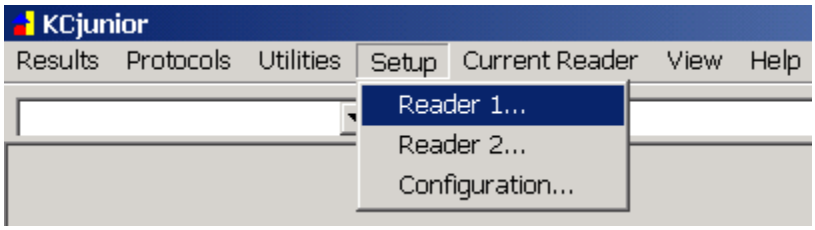


Figure 21: KCjunior main menu, Setup|Reader 1 dialog

3. The **Reader Setup 1** dialog will appear. Select ELx800,EL800 at **Reader Type**, and define the communication parameters.Click **OK** to return to the main menu screen.

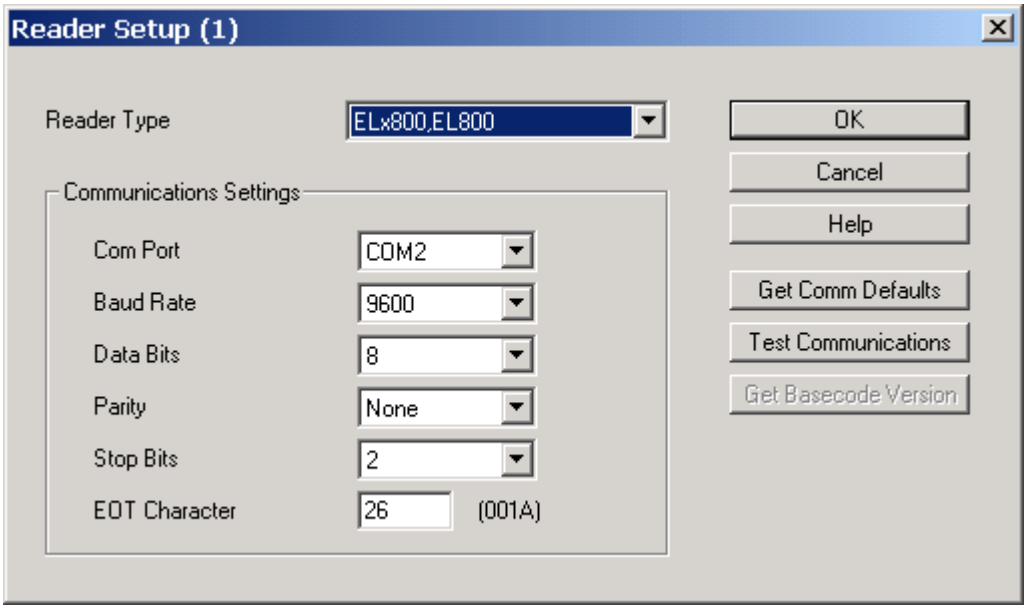


Figure 22: Reader Setup 1 dialog

4. Click **Read Plate** to enter the **Results ID** and **Plate Description**.

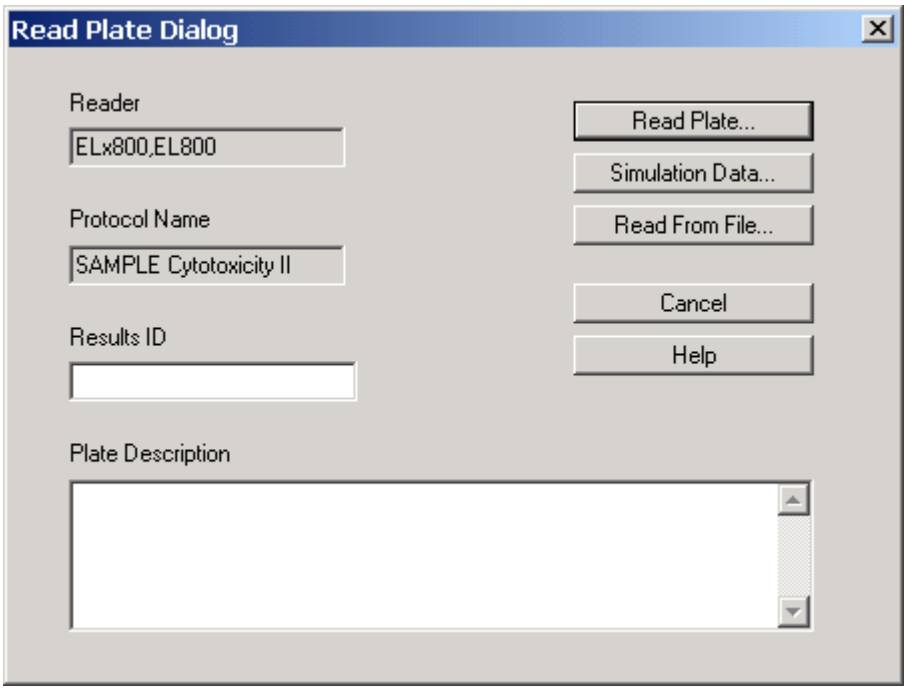


Figure 23: Read Plate dialog

5. Choose **New Protocol** to define the protocol.

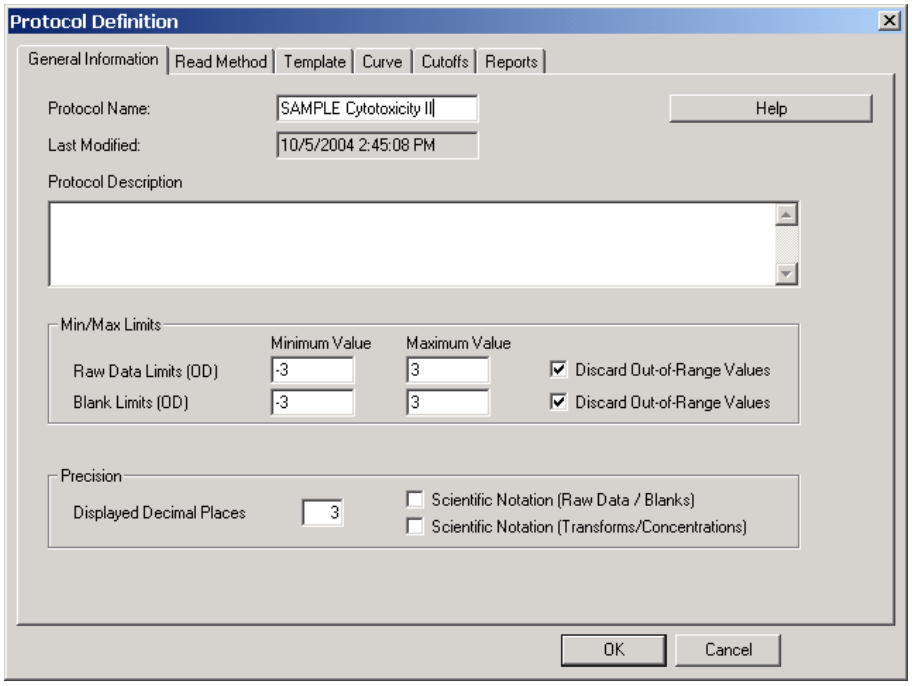


Figure 24: Protocol Definition (General Information) dialog

6. Define the **Read Method** parameters.
   - Set the **Plate Geometry** to **8 x 12**.

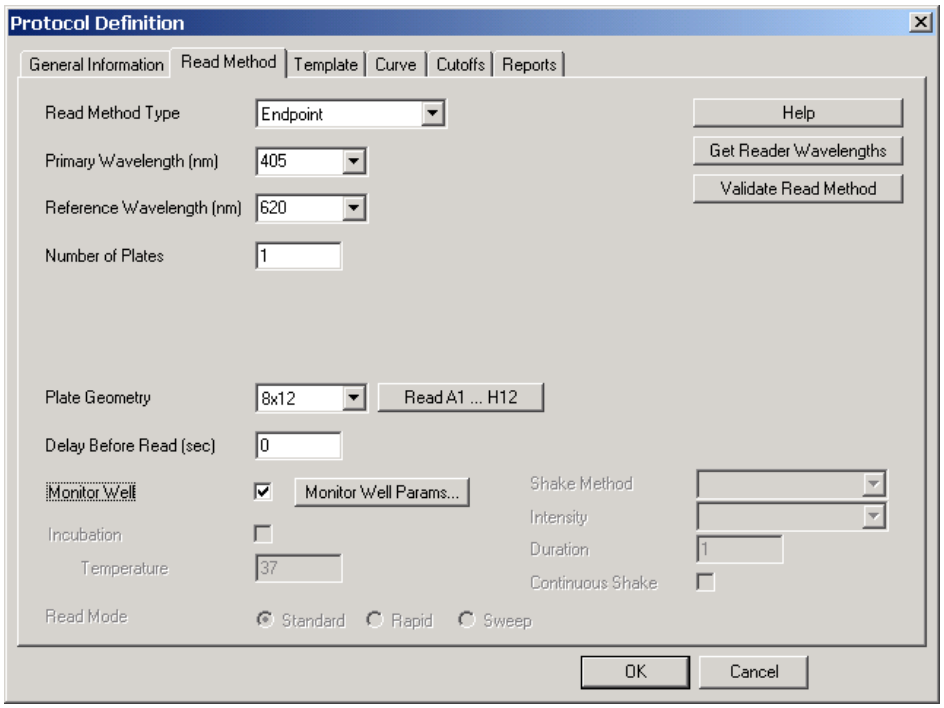


Figure 25: Protocol Definition (Read Method) dialog

7. Click **OK** to read the plate and display the results.

KC4™

Bio-Tek's KC4 data reduction package can control the ELx800 in 6-, 12-, 24-, 48-, 96, and 384-well formats, as well as in 60-, 72-, and 96-well Terasaki plate formats.

1. Start KC4.

2. At KC4's main menu, choose **System|Readers**.

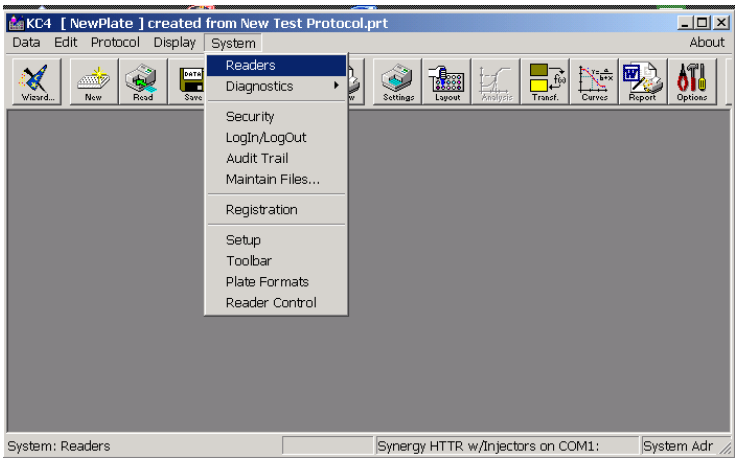


Figure 26: KC4 main menu, System|Readers

3. The **Reader Selection** dialog will appear. Select the ELx800 reader and define the communications parameters. Click on **Close** to return to the main menu.

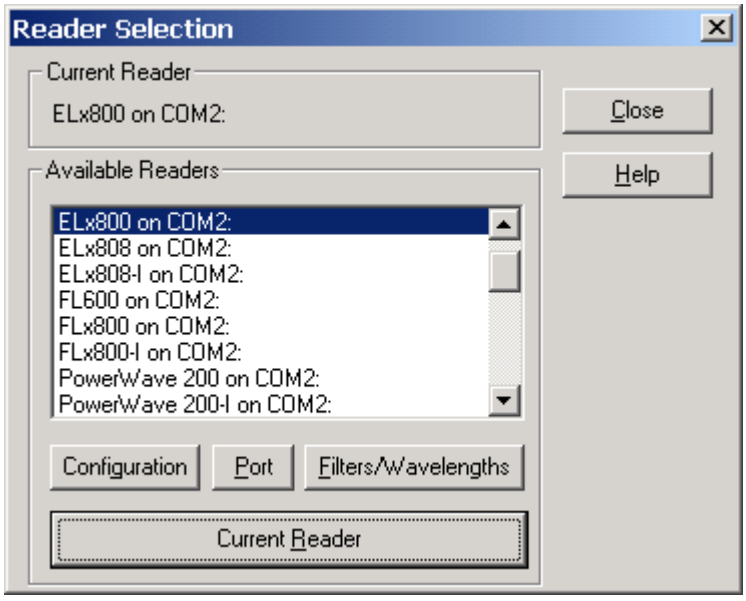


Figure 27: Reader Selection dialog

4. Select **Data|New Plate**.

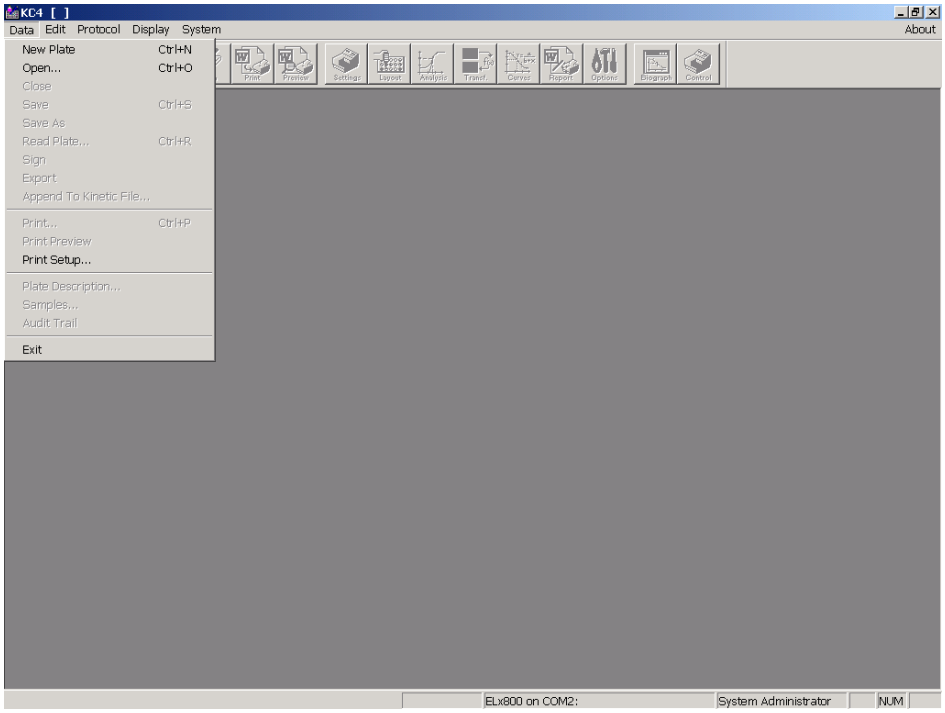


Figure 28: Data|New Plate dialog

5. If prompted to select a protocol, select "Empty Protocol" and click **OK**. If not prompted, select **Protocol|New**, or use KC4's Protocol Wizard to step through protocol creation.

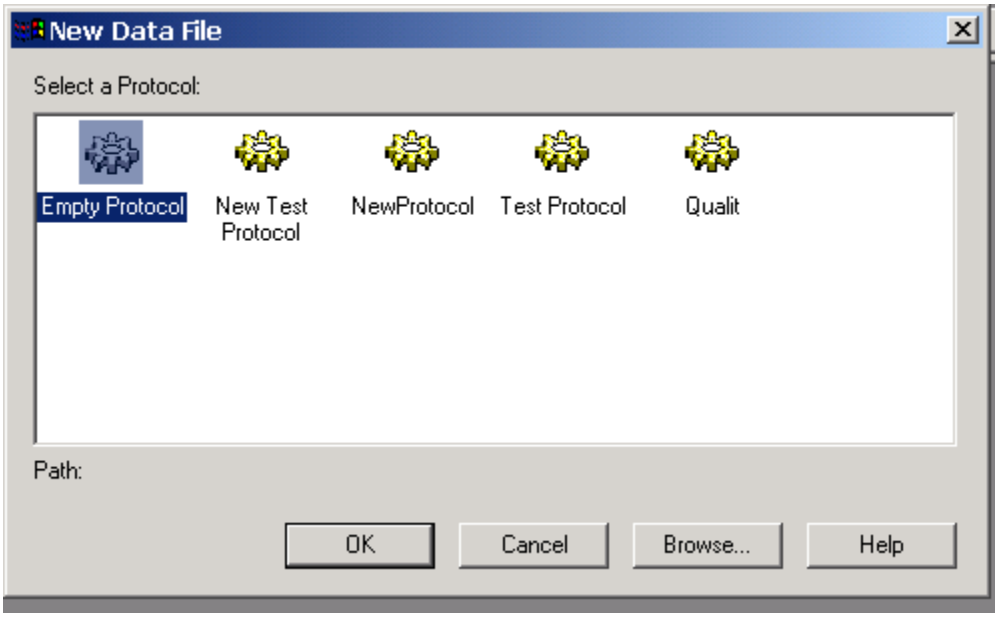


Figure 29: New Data File dialog

6. Select **Protocol|Reading** from the main menu. The **Reading parameters** dialog will appear.

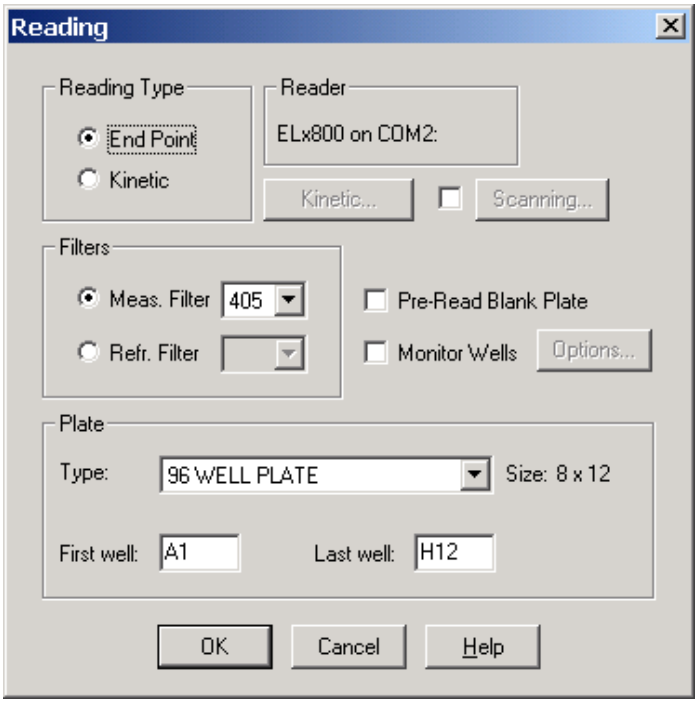


Figure 30: Reading Parameters dialog

7. Select a Reading Type of **End Point** or **Kinetic**.
8. Define the wavelength(s) at which the plate will be read.
9. Select a Plate Type.
10. Define other reading parameters as necessary. Click the Help button for assistance.
11. When complete, click **OK**.
12. Select **Data|Read Plate**. The Plate Reading dialog will appear.
13. Enter any comments, place the plate on the carrier, then click **Start Reading** to begin the plate read.
    - The plate will be read and then the raw data results will display in KC4.
    - To analyze, manipulate, or print results, **protocol** parameters should be defined. Refer to KC4's Help system or User's Guide for instructions.

Appendix C

Report Format

This appendix contains examples of reports that can be generated and/or printed from the ELx800. Refer to **Printing Reports** in **Chapter 3** for details on how to print these reports. In addition, an Assay List, Assay Definition, Map, and Result can be printed by choosing Report from the Main Menu screen.

Bio-Tek Instruments

Assay:Open Assay 04
Wavelength:405

Date:03/16/04
Time:01:05:34PM
Temp:

Lot: _____
Operator:
Plate ID:

COMMENTS

	1	2	3	4	5	6	7	8	9	10	11	12
A												
CALL	VAL	POS	POS	POS	NEG	POS	EQUIV	NEG	NEG	POS	POS	POS
CalcOD	0.109	0.085	0.090	0.610	1.501	0.112	0.846	1.465	2.524	0.216	0.187	0.279
Well	PC	SMP7	SMP15	SMP23	SMP31	SMP39	SMP47	SMP55	SMP63	SMP71	SMP79	SMP87
RSLT												
B												
CALL	VAL	EQUIV	POS	POS	NEG	EQUIV	NEG	EQUIV	POS	POS	NEG	NEG
CalcOD	0.119	1.054	0.091	0.105	1.672	1.007	1.939	0.828	0.715	0.372	1.675	1.625
Well	PC	SMP8	SMP16	SMP24	SMP32	SMP40	SMP48	SMP56	SMP64	SMP72	SMP80	SMP88
RSLT												
C												
CALL	POS	EQUIV	POS	POS	NEG	EQUIV	POS	POS	NEG	POS	NEG	POS
CalcOD	0.290	1.092	0.243	0.109	2.291	1.000	0.127	0.470	2.129	0.218	1.636	0.372
Well	SMP1	SMP9	SMP17	SMP25	SMP33	SMP41	SMP49	SMP57	SMP65	SMP73	SMP81	SMP89
RSLT												
D												
CALL	POS	NEG	POS	POS	NEG	NEG	POS	POS	POS	NEG	NEG	POS
CalcOD	0.301	2.075	0.237	0.133	2.643	2.032	0.635	0.663	0.198	1.656	1.626	0.523
Well	SMP2	SMP10	SMP18	SMP26	SMP34	SMP42	SMP50	SMP58	SMP66	SMP74	SMP82	SMP90
RSLT												
E												
CALL	NEG	NEG	NEG	POS	POS	NEG	POS	POS	POS	POS	EQUIV	POS
CalcOD	2.205	2.064	2.473	0.092	0.134	2.023	0.149	0.254	0.658	0.244	0.829	0.488
Well	SMP3	SMP11	SMP19	SMP27	SMP35	SMP43	SMP51	SMP59	SMP67	SMP75	SMP83	SMP91
RSLT												
F												
CALL	NEG	NEG	NEG	POS	POS	NEG	POS	POS	NEG	POS	EQUIV	POS
CalcOD	2.811	3.214	2.381	0.651	0.589	3.856	0.283	0.753	3.803	0.355	0.880	0.328
Well	SMP4	SMP12	SMP20	SMP28	SMP36	SMP44	SMP52	SMP60	SMP68	SMP76	SMP84	SMP92
RSLT												
G												
CALL	NEG	NEG	NEG	POS	POS	NEG	POS	POS	POS	EQUIV	POS	POS
CalcOD	3.499	3.663	2.857	0.088	0.183	3.776	0.150	0.207	0.213	1.151	0.260	0.215
Well	SMP5	SMP13	SMP21	SMP29	SMP37	SMP45	SMP53	SMP61	SMP69	SMP77	SMP85	SMP93
RSLT												
H												
CALL	NEG	EQUIV	NEG	POS	POS	POS	POS	POS	POS	POS	POS	POS
CalcOD	3.225	1.045	2.889	0.090	0.149	0.100	0.532	0.158	0.712	0.225	0.721	0.238
Well	SMP6	SMP14	SMP22	SMP30	SMP38	SMP46	SMP54	SMP62	SMP70	SMP78	SMP86	SMP94
RSLT												

Figure 31: Samples with calls on Matrix Report

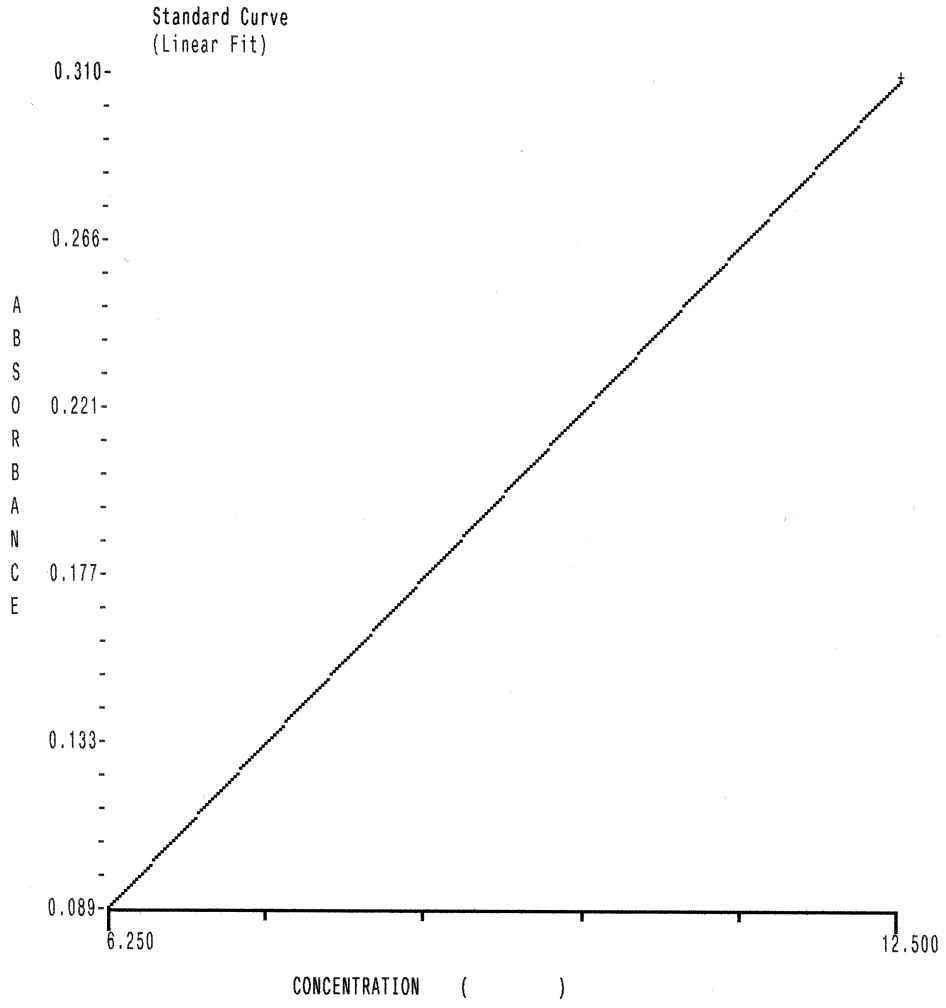
Bio-Tek Instruments

Assay:ASSAY 01
Wavelength:450

Date:03/16/04
Time:02:27:57PM
Temp:

Lot: _____
Operator:
Plate ID:

COMMENTS



Curve: y = Ax + B
Coefficients: A=0.035 B=-.132
R-Sqr: 1.0000

Figure 32: Curve Fit Report

Bio-Tek Instruments

Assay:Open Assay 04 Date:03/16/04 Lot: _____
Time:01:05:34PM Operator:
Wavelength:405 Temp: Plate ID:

COMMENTS

Interpretation of Results

GREYZONE=0.20 =0.200
CUTOFF=1 =1.000

SMP : SMP<=(CUTOFF+(CUTOFF*GREYZONE))AND(SMP>=(CUTOFF-(CUTOFF*GREYZONE))) : EQUIV
SMP : SMP>(CUTOFF+(CUTOFF*GREYZONE)) : NEG
SMP : SMP<(CUTOFF-(CUTOFF*GREYZONE)) : POS

PC : PC > .01

Well	ID	BlkdOD	CalcOD	Call	Pred Conc	RSLT	Std Dev	CV%	Notes:
A01	PC	0.109	0.109	VAL					
B01	PC	0.119	0.119	VAL					
AVE		0.114	0.114				0.007	6.163	
C01	SMP0001	0.290	0.290	POS					
D01	SMP0002	0.301	0.301	POS					
E01	SMP0003	2.205	2.205	NEG					
F01	SMP0004	2.811	2.811	NEG					
G01	SMP0005	3.499	3.499	NEG					
H01	SMP0006	3.225	3.225	NEG					
A02	SMP0007	0.085	0.085	POS					
B02	SMP0008	1.054	1.054	EQUIV					
C02	SMP0009	1.092	1.092	EQUIV					
D02	SMP0010	2.075	2.075	NEG					
E02	SMP0011	2.064	2.064	NEG					
F02	SMP0012	3.214	3.214	NEG					
G02	SMP0013	3.663	3.663	NEG					
H02	SMP0014	1.045	1.045	EQUIV					
A03	SMP0015	0.090	0.090	POS					

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Figure 33: Samples with calls on Column Report

Bio-Tek Instruments

Assay:Open Assay 04 Date:03/16/04 Lot: _____
Time:01:05:34PM Operator:
Wavelength:405 Temp: Plate ID:

COMMENTS

Interpretation of Results

GREYZONE=0.20 =0.200
CUTOFF=1 =1.000

SMP : SMP<=(CUTOFF+(CUTOFF*GREYZONE))AND(SMP>=(CUTOFF-(CUTOFF*GREYZONE))) : EQUIV
SMP : SMP>(CUTOFF+(CUTOFF*GREYZONE)) : NEG
SMP : SMP<(CUTOFF-(CUTOFF*GREYZONE)) : POS

PC : PC > .01

Well ID	BlkdOD	CalcOD	Call	Pred Conc	RSLT	Std Dev	CV%	Notes:
A01 PC	0.109	0.109	VAL					
B01 PC	0.119	0.119	VAL					
AVE	0.114	0.114				0.007	6.163	

End of Report

Figure 34: Column Report without samples

Bio-Tek Instruments

Panel

Date:07/31/96 Lot: _____
Time:02:24:01PM Operator:
Temp: Plate ID:

COMMENTS

Specimen	Assay	Well	BlkdOD	CalcOD	Call	RSLT	Std Dev	CV%	Notes:
SMP0001	ASSAY 01	F01	-.006	-.006					
	ASSAY 02	B05	0.000	0.000					
SMP0002	ASSAY 01	G01	0.002	0.002					
	ASSAY 02	C05	0.007	0.007					
SMP0003	ASSAY 01	H01	0.005	0.005					
	ASSAY 02	D05	0.004	0.004					
SMP0004	ASSAY 01	A02	0.002	0.002					
	ASSAY 02	E05	0.012	0.012					
SMP0005	ASSAY 01	B02	-.002	-.002					
	ASSAY 02	F05	0.010	0.010					
SMP0006	ASSAY 01	C02	0.005	0.005					
	ASSAY 02	G05	-.001	-.001					
SMP0007	ASSAY 01	D02	-.001	-.001					
	ASSAY 02	H05	0.002	0.002					
SMP0008	ASSAY 01	E02	0.009	0.009					
	ASSAY 02	A06	0.004	0.004					
SMP0009	ASSAY 01	F02	0.006	0.006					
	ASSAY 02	B06	0.007	0.007					
SMP0010	ASSAY 01	G02	-.006	-.006					
	ASSAY 02	C06	0.010	0.010					
SMP0011	ASSAY 01	H02	-.005	-.005					
	ASSAY 02	D06	0.004	0.004					
SMP0012	ASSAY 01	A03	-.00	-.00					
	ASSAY 02	E06	0.003	0.003					
SMP0013	ASSAY 01	B03	0.002	0.002					
	ASSAY 02	F06	-.001	-.001					
SMP0014	ASSAY 01	C03	-.005	-.005					
	ASSAY 02	G06	0.001	0.001					
SMP0015	ASSAY 01	D03	-.002	-.002					

Page 1

Figure 35: Panel Report

01:47PM 03/16/04

NAME:	Open Assay 04
READ TYPE:	ENDPOINT
PLATE TYPE:	96
WAVELENGTH:	405
BLANK MAP:	AIR
CURVE-FIT TYPE:	LINEAR
EDIT STD OUTLIERS:	NONE
X/Y AXIS TYPE:	LIN
EXTRAPOLATE UNKNOWNS?	NO

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP
B	PC	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP
C	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP
D	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP
E	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP
F	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP
G	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP
H	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP	SMP

Figure 36: Assay Detail Report (Sheet 1 of 2)

Assay List
Version: 3.7

1	ASSAY	01
2	ASSAY	02
3	ASSAY	03
4	ASSAY	04
5	ASSAY	05
6	ASSAY	06
7	ASSAY	07
8	ASSAY	08
9	ASSAY	09
10	ASSAY	10
11	ASSAY	11
12	ASSAY	12
13	ASSAY	13
14	ASSAY	14
15	ASSAY	15
16	ASSAY	16
17	ASSAY	17
18	ASSAY	18
19	ASSAY	19
20	ASSAY	20
21	ASSAY	21
22	ASSAY	22
23	ASSAY	23
24	ASSAY	24
25	ASSAY	25
26	ASSAY	26
27	ASSAY	27
28	ASSAY	28
29	ASSAY	29
30	ASSAY	30
31	ASSAY	31
32	ASSAY	32
33	ASSAY	33
34	ASSAY	34
35	ASSAY	35
36	ASSAY	36
37	ASSAY	37
38	ASSAY	38
39	ASSAY	39
40	ASSAY	40
41	ASSAY	41
42	ASSAY	42
43	ASSAY	43
44	ASSAY	44
45	ASSAY	45
46	ASSAY	46
47	ASSAY	47
48	ASSAY	48
49	ASSAY	49
50	ASSAY	50
51	ASSAY	51
52	ASSAY	52
53	ASSAY	53
54	ASSAY	54
55	ASSAY	55

Figure 37: Assay Detail Report (Sheet 2 of 2)

Appendix D

Comparison of the ELx800 and the EL800

The following chart compares the features on the ELx800 and EL800 microplate readers.

Feature	ELx800	EL800
340-750 nm		
405-750 nm	x	x
Single- and dual-wavelength reading	x	x
6-well plates	x	
12-well plates	x	
24-well plates	x	
48-well plates	x	
96-well plates	x	x
Edit Assay Name	x	
Absorbance Values	x	x
Formulas	x	
Curve fits	x	
# Assays available	55	10
# Results stored	8	1
Blanks and samples only in Map		x
Blanks, standards, controls and blanks in Map	x	
Panel Assay	x	
Computer Control capability*	x	x
Serial and Parallel output	x	x
Customizable via **Extensions®**	x	

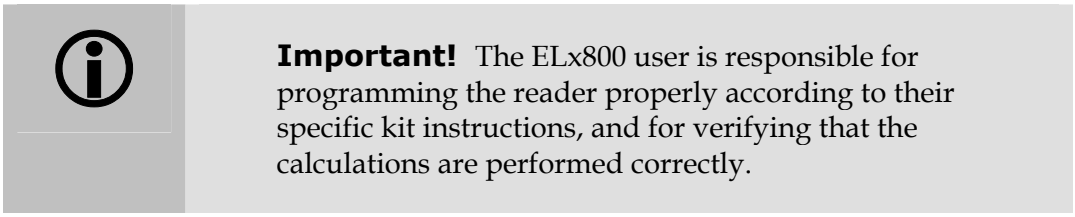
***The **EL800** version can be computer controlled and will function as an ELx800.

All **ELx800** optical specifications apply to the ELx800.

Appendix E

Instructions for Programming a New Assay

This appendix provides two examples of assay kit instructions and step-by-step directions for programming each assay. The appendix includes two sample assays: one with a ratio transformation calculation and a POS/NEG cutoff determination, and another with a standard curve.These examples are based on real assays; however, the results have not been verified on any clinical kits. The kit instructions are provided so that users can see how it is possible to translate the kit wording into an ELx800 assay program. For clarity, only the user menu choices from the reader screens are shown. Refer to **Chapter 3** for details.



Important! The ELx800 user is responsible for programming the reader properly according to their specific kit instructions, and for verifying that the calculations are performed correctly.

Sample ANA Screen Enzyme Immunoassay Kit (Transformation and Cutoff)

Intended Use

This assay is designed for the in vitro screening of human serum for the presence of specific IgG antinuclear antibodies (ANAs), to aid in diagnosing certain systemic rheumatic diseases. Sufficient materials are supplied to allow a maximum of 93 samples to be screened in single, with a positive, cutoff, and negative controls.

Background

Antinuclear antibodies occur in a large number of patients with systemic rheumatic diseases. These diseases are characterized by the presence of one or more ANAs.

Sera positive on this ANA kit should be tested for the specific autoantibodies indicative of the various systemic rheumatic diseases.

Principle of the Assay

Microwells are pre-coated with purified antigens. The prediluted controls and diluted patient samples are added to the wells, and autoantibodies recognizing one or a combination of antigens bind during the first incubation. After washing the wells to remove all unbound proteins, IgG conjugate is added. The conjugate binds to the captured human autoantibody, and the excess unbound conjugate is removed by a further wash step. Substrate is added that causes a blue reaction, thereby exposing the bound conjugate, and producing an intensity proportional to the concentration of autoantibody in the sample. Phosphoric acid is added to each well to stop the reaction. This produces a yellow end-point color, which is read at 450 nm.

Materials

Materials Supplied

- **Instruction Leaflet:** Giving full assay details.
- **QC Certificate:** Indicating the expected performance of the batch.
- **ANA Coated Wells:** 12 x 8 well strips coated with purified antigens.
- **Type III Wash Buffer 20x Concentrate:** 1 bottle containing 50 ml of a concentrated buffer for washing the wells.
- **Type III Sample Diluent:** 2 bottles containing 50 ml of buffer for sample dilution. Ready to use.
- **ANA Positive Control:** 1 bottle containing 1.8 ml of diluted stabilized human serum. Ready to use.
- **ANA Cutoff Control:** 1 bottle containing 1.8 ml of diluted stabilized human serum. Ready to use.
- **ANA Negative Control:** 1 bottle containing 1.8 ml of diluted stabilized human serum. Ready to use.
- **ANA Conjugate:** 1 bottle containing 12 ml of peroxidase labeled antibody to human IgG. Colored red, ready to use.
- **TMB Substrate:** 1 bottle containing 14 ml TMB substrate. Ready to use.
- **Stop Solution:** 1 bottle containing 14 ml of 3M Phosphoric acid. Ready to use.

Additional Materials and Equipment – Not Supplied

- **Automatic Microplate Plate Washer:** This is recommended; however, plate washing can be performed manually.
- **Plate Reader:** Capable of measuring optical densities at 450 nm referenced on air.
- **Distilled or Deionized Water:** This should be of the highest quality available.
- **Calibrated Micropipettes:** For dispensing 1000, 100, and 10 µl.
- **Multichannel Pipette:** Recommended for dispensing 100 µl volumes of conjugate, substrate, and stop solution.
- **Glass/Plastic Tubes:** For sample dilution.

Quality Control and Results

Quality Control

For an assay to be valid, all the following criteria must be met:

- Cutoff as well as Positive and Negative controls must be included in each run.
- The OD of the cutoff and the ANA result of the Negative and Positive Controls should be in the ranges specified on the QC Certificate.
- For example: The absorbance of the Positive Control must be greater than 1.200 OD.

  The absorbance of the Negative Control must be less than 0.300 OD.

❖ **Note:** If the above criteria are not met, the assay is invalid and the test should be repeated.

Calculation of the Sample Results

Use the following formula to calculate the ANA result for each sample:

$$\frac{\text{Control or sample OD}}{\text{Cutoff control OD}} \times 10 = \text{Control or sample value (U/ml)}$$

Expected Values

The normal range was determined on serum from 200 normal adult blood donors. The cutoff control has been set at a point equivalent to the upper normal limit, or a cutoff level of 10 U/ml.

The ranges are provided as a guide only. ELISA assays are very sensitive and capable of detecting small differences in sample populations. It is recommended that each laboratory determine its own normal range, based on the population techniques and equipment employed.

ANA Result	Interpretation
< 10.0	Negative
> 10.0	Positive

Programming the ANA Screen Enzyme Immunoassay Kit (Transformation and Cutoff)

From the Main Menu, press DEFINE. Select the assay name and edit the name if desired. At the DEFINE menu:

STEP	COMMENTS
To program the reading method, press:	
METHOD	
WAVELENGTH (Dual or Single): Single	
MEASURE (Wavelength): 450	
PLATE TYPE: 96	
To program the plate map, press:	
MAP	
AUTO:	"Auto" mapping is normally preferred, because it fills in the well IDs logically and automatically after determining which direction to map and how many wells to fill.
DOWN:	Maps the wells down the column
DOWN:	Locates replicates in a vertical orientation down the column
A01:	Begins mapping at well location A01
BLANK MAP: AIR	Choose to blank on "AIR" if no blank wells are required
NUMBER STDS: 00	
NUMBER CTLS: 03	
CONTROL 1: PC	
CONTROL 2: CTL1	Suggested choice for the cutoff control
CONTROL 3: NC	
NUMBER OF REPLICATES	
PC: 01	
CTL1: 03	

STEP	COMMENTS
SAMPLES: 91	User-defined; recommendation is to fill the entire plate
SAMPLE REPLICATES: 01	
Use the MAP and MATH keys to create the control validation formula:	
FORMULA	
VAL	
CONTROL:	When defining control validation formulas, use "PC" to indicate the criterion for each of the PC replicates, and "PC;x" to indicate the average of the Positive Control replicates.
PC;x > 1.200	
NO. OF REPLICATES: 01	
NC;x < 0.300	
NO. OF REPLICATES: 01	
To create the plate transformation for CALC, divide the ANA result by a cutoff standard.	
FORMULA	
*MORE	
TRANS-VAR	
SCOPE VARIABLE (SMP or OD)	Select OD to advance to the formula definition screen and define the transformation variable (Tvar)
FORMULA	
CTL1;X	Defining TRANS VAR = CTL1;X isolates the OD value for CTL1;X for use in transformation
TRANS:	
FORMULA: (OD/TVAR)*10	Converts all OD values on plate to "ANA Result" per kit insert instructions
To define a cutoff formula for Positive and Negative calls:	
FORMULA	
CUTOFF: 10.0	
GREYZONE: 00%	
SAMPLE > CUTOFF + 00%: POS	

Sample Human Anticardiolipin IgG Enzyme Immunoassay Kit
(Standard Curve and Cutoff)

Intended Use

This assay is intended for the in vitro measurement of IgG anticardiolipin antibodies in human serum, as an aid in the diagnosis of antiphospholipid syndrome (APS).

Sufficient materials are supplied to allow a maximum of 41 samples to be tested in duplicate or 89 in single, with a standard curve and positive and negative controls.

Background

Anticardiolipin antibodies are found in a wide range of conditions either transiently, in some infectious diseases, or more persistently in autoimmune diseases such as systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS). Anticardiolipin antibodies have also been associated with a range of clinical conditions including fetal loss, endocarditis, stroke, heart attack and autoimmune haemolytic.

Principle of the Assay

Microwells are precoated with cardiolipin and cofactor. Standards, controls, and patient samples are added to the wells, and autoantibodies recognizing cardiolipin bind during the first incubation. After washing the wells to remove all unbound proteins, conjugate is added. The conjugate binds to the captured human antibody, and the excess unbound conjugate is removed by a further wash step. Substrate is added that causes a blue reaction, thereby exposing the bound conjugate and producing an intensity proportional to the concentration of autoantibody in the sample. Phosphoric acid is added to each well to stop the reaction. This produces a yellow end-point color, which is read at 450 nm.

Materials

Materials Supplied

- **Instruction Leaflet:** Giving full assay details.
- **QC Certificate:** Indicating the expected performance of the batch.
- **Cardiolipin Coated Wells:** 12 break-apart 8-well strips coated with bovine cardiolipin antigen. The plate is packaged in a re-sealable foil bag containing two desiccant pouches.
- **Type II Sample Diluent:** 2 bottles containing 50 ml of buffer for sample dilution. Colored yellow, ready to use.
- **Type II Wash Buffer (20x Concentrate):** 1 bottle containing 50 ml of a 20-fold concentrated buffer for washing the wells.
- **Cardiolipin IgG Standards:** 5 bottles each containing 1.2 ml of diluted human serum, with the following concentrations of anticardiolipin autoantibody: 100, 50, 25, 12.5, 6.25 GPL U/ml. Ready to use.

  The standard set is calibrated against the Louisville APL reference preparation.

- **Cardiolipin IgG Positive Control:** 1 bottle containing 1.2 ml of diluted human serum. The expected value is given on the QC certificate. Ready to use.
- **Cardiolipin Negative Control:** 1 bottle containing 1.2 ml of diluted human serum. The expected value is given on the QC certificate. Ready to use.
- **Cardiolipin IgG Conjugate:** 1 bottle containing 12 ml of purified peroxidase labeled antibody. Colored red, ready to use.
- **TMB Substrate:** 1 bottle containing 14 ml TMB substrate. Ready to use.
- **Stop Solution:** 1 bottle containing 14 ml of 3M phosphoric acid. Ready to use.

Additional Materials and Equipment – Not Supplied

- **Automatic Microplate Plate Washer:** This is recommended; however, plate washing can be performed manually.
- **Plate Reader:** Capable of measuring optical densities at 450 nm referenced on air.
- **Distilled or Deionized Water:** This should be of the highest quality available.
- **Calibrated Micropipettes:** For **dispensing** 1000, 100, and 10 µl.
- **Multichannel Pipette:** Recommended for dispensing 100 µl volumes of conjugate, substrate, and stop solution.
- **Glass/Plastic Tubes:** For sample dilution.

Quality Control and Results

Quality Control

For an assay to be valid, all the following criteria must be met:

- Standards and the positive and negative controls must be included in each run.
- The values obtained for all the controls should be in the ranges specified on the QC Certificate.
- The curve shape should be similar to the standard curve, shown on the QC Certificate.
- If the above criteria are not met, the assay is invalid and the test should be repeated.

Calculate Mean Optical Densities (for assays run in duplicate only)

For each standard, control and sample calculate the mean OD of the duplicate readings. The user must verify that the percentage coefficient of variation (%CV) for each duplicate OD is less than 15.0%.

Plot Calibration Curve

The calibration curve can be plotted either automatically or manually as follows by plotting the anticardiolipin autoantibody concentration on the log scale against the OD on the linear scale for each calibrator:

- Automatic - Use appropriately validated software, and the curve fit that best fits the data.
- Manual - Using log/linear graph paper, draw a smooth curve through the points (not a straight line or point to point).

Treatment of Anomalous Points

If any one point does not lie on the curve, it can be removed. If the absence of this point means that the curve has a shape dissimilar to that of the sample calibration curve, or more than one point appears to be anomalous, then the assay should be repeated.

Calculation of Autoantibody Levels in Controls and Samples

Read the level of the anticardiolipin autoantibody in the controls and diluted samples directly from the calibration curve. The control values should fall within the range given on the QC Certificate.

❖ **Note:** The standard values have been adjusted by a factor of 100 to account for a 1:100 sample dilution. No further correction is required.

Assay Calibration

The assays are calibrated against the Louisville reference LAPL-GM-100. One GPL unit is defined as the cardiolipin binding activity of 1 µg/ml of an affinity purified IgG anticardiolipin preparation from a standard serum.

The Louisville reference center recommends the following positive discrimination criteria according to the recommendation of the 2nd International Anticardiolipin Workshop.

Criteria Range	(GPLU/ml)
High Positive	> 80
Medium Positive	≥ 20-80
Low Positive	≥ 10, < 20

Results Interpretation

The association between low positive levels of anticardiolipin antibodies and clinical findings is unclear.

Normal population studies indicate that there is a higher prevalence of IgM positives in the normal population than IgG, 9.4% and 6.5%, respectively. In normal pregnancy, the levels are higher still at 17.0% (IgM) and 10.6% (IgG).

Expected Values

The normal range was determined on serum from 102 normal adult blood donors. The ranges below are provided as a guide only. ELISA assays are very sensitive and capable of detecting small differences in sample populations. It is recommended that each laboratory determine its own normal range, based on the population techniques and equipment employed.

IgG Anticardiolipin	
< 11 GPL U/ml	Negative result
> 11 GPL U/ml	Positive result

Programming the Human Anticardiolipin IgG Enzyme Immunoassay Kit (Standard Curve and Cutoff)

From the Main Menu, press **DEFINE**. Select the assay name and edit the name if desired. At the define menu, follow steps below:

STEP	COMMENTS
To program the reading method, press:	
METHOD	
WAVELENGTH (Dual or Single): Single	
MEASURE (Wavelength[s] to use): 450	
PLATE TYPE: 96	
To program the plate map, press:	
MAP	
AUTO	
DOWN	
DOWN	
A01	
BLANK MAP: AIR	
NUMBER STDS: 05	
NUMBER STD REPLICATES: 01	
CONCENTRATIONS: STD1: 6.25 STD2: 12.5 STD3: 25 STD4: 50 STD5: 100	
NUMBER CTLS: 02	
CONTROL 1: PC	
CONTROL 2: NC	
NUMBER OF REPLICATES	
PC: 01	
NC: 01	
SAMPLES: 89	
SAMPLE REPLICATES: 01	

STEP	COMMENTS
To define a cutoff formula for Positive and Negative calls:	Kit instructions specify that samples with concentration values greater than 11 should appear as positive. The ELx800 software calculates the cutoff based on absorbance value or transformed value (see the previous example) and cannot calculate based on concentration. The technician must make the positive or negative determination visually, based on the calculated concentration results.
Curve: 4P	As a general guideline, choose “linear” if you expect a straight line result. Choose “4P” for all others, unless otherwise specified by the kit instructions. The reader will automatically calculate the concentrations of the samples when the assay is run.